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1. Whitelaw, J.W.: Hypoparathyroidism in Children. *Bull. Vancouver Med. Assn.*, 30:116, Dec., 1953.
2. Sandock, Isadore: Tetany and Ovarian Function. *J.A.M.A.*, 160:659, Feb. 25, 1956.
3. Grollman, Arthur: *Essentials of Endocrinology*. Philadelphia, J. B. Lippincott Co., 2nd ed., 1947, p. 269.

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The American Journal of Medicine

Vol. XXVII DECEMBER 1959 No. 6

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Clinical Studies

- Stimulation of the Carotid Sinus in Man. I. The Cerebral Response. II. The Signifi-
cance of Head Positioning
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This is a careful study of what has become known as carotid sinus reflex hypersensitivity, which demonstrates quite convincingly some of the flaws in the testing and interpretation of this syndrome. It is evident that carotid artery compression rather than carotid sinus stimulation is the usual test incitant, that head position is an important factor, and that no true reflex is involved in most cases. Many cases really fall in the category of carotid artery insufficiency. A unilaterally "sensitive" sinus was found usually to signify stenosis or occlusion of the *opposite* internal carotid.

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NEW—DELICIOUS—ORANGE-FLAVORED PENTIDS "400" FOR SYRUP

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Studies on the Nature of the Increased Serum Acid Phosphatase in Gaucher's Disease

LESTER R. TUCHMAN, GILBERT GOLDSTEIN AND MARTIN CLYMAN 959

It is perhaps not sufficiently appreciated that a modest rise in serum acid phenylphosphatase occurs in patients with Gaucher's disease, and that this finding may be of considerable aid in the diagnosis of that disorder. The present study indicates that the enzyme in question may be differentiated from prostatic and erythrocyte acid phosphatases by its resistance to L-tartrate, formaldehyde and copper salts, thus further refining the value of this test in differential diagnosis.

Hypoproteinemia Antedating Intestinal Lesions, and Possibly Due to Excessive Serum Protein Loss into the Intestine

HALSTED HOLMAN, WILLIAM F. NICKEL, JR. AND MARVIN H. SLEISENGER 963

This unusually interesting study, concerned with a newly recognized pathogenesis of "idiopathic" hypoproteinemia (loss into the gastrointestinal tract), makes a number of significant points. By carefully controlled use of I^{131} -labeled albumin and gamma globulin, plasma proteins are unequivocally demonstrated to appear in substantial amounts in the intestinal juice, both in normal subjects and in patients with idiopathic hypoproteinemia. Serum albumin synthesis in these patients is shown not to be in excess of normal, from which it may be inferred (although the data do not conclusively demonstrate this) that the hypoproteinemia is the result of excessive exudation or secretion into the gut. Protracted observation of the patients disclosed that while at first gastrointestinal lesions could not be demonstrated in association with the idiopathic hypoproteinemia, one or another type of inflammatory lesion ultimately became apparent. One may anticipate further developments of great clinical and metabolic interest in this area.

Seminar on Mycotic Infections

Cryptococcosis (Torulosis). Current Concepts and Therapy . . . M. L. LITTMAN 976

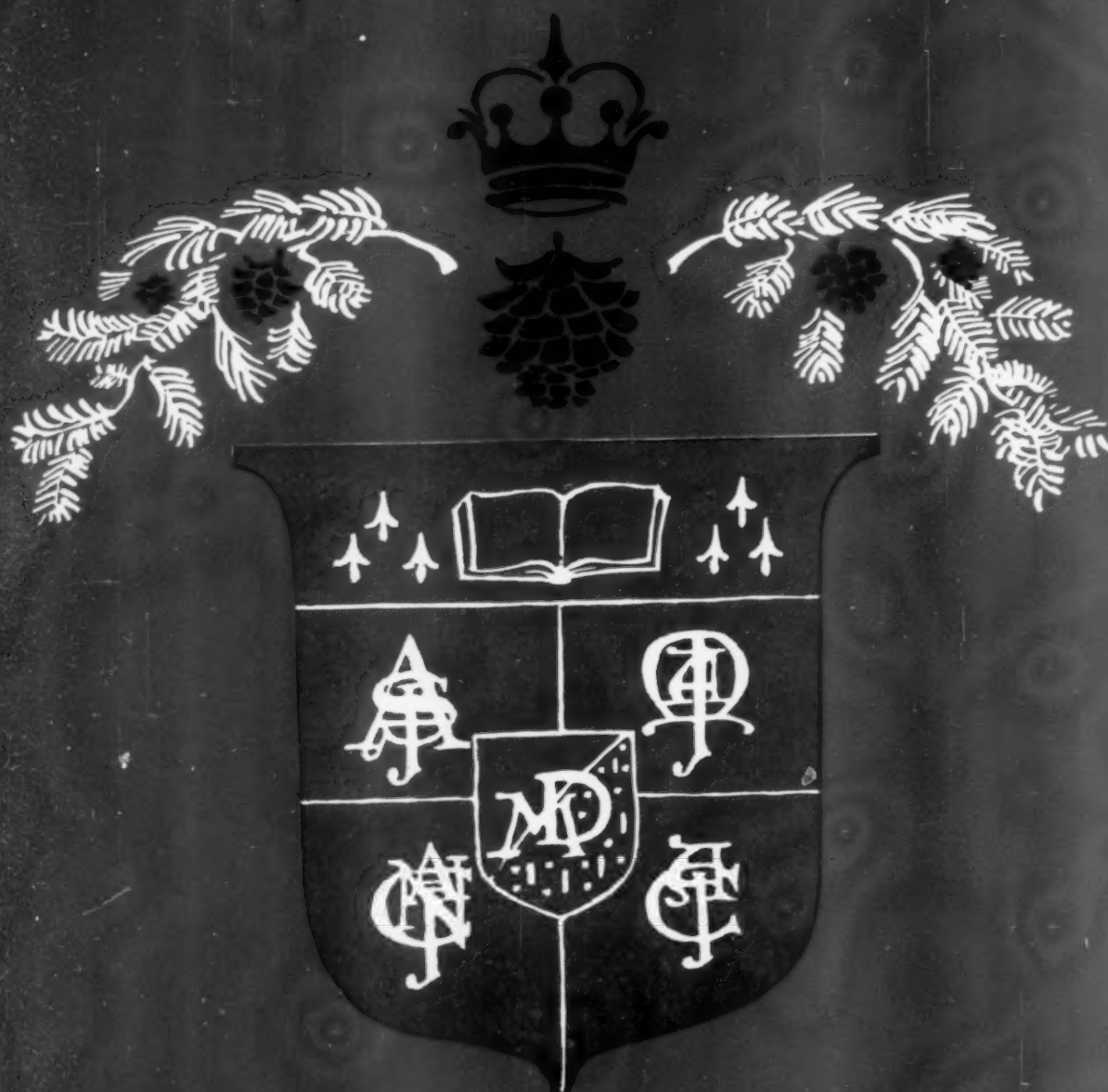
The discussion begins with a consideration of the clinical and pathologic features of cryptococcosis, notably of the disseminated disease with meningoencephalitis (more commonly than ever associated with lymphomas and leukemias), also of pulmonary cryptococcosis (now more frequently recognized following inhalation of pigeon excreta dust). There follow sections on epidemiology and laboratory diagnosis, definitive only when the fungus is cultured from the suspected site. There is a full discussion of the metabolic requirements and biosynthetic pathways of *C. neoformans*. The concluding chapter deals with treatment, with special reference to the use of amphotericin B.

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The third investigator reports: "From our own clinical observations it would appear that deserpidine is the most desirable compound among those presently available."³

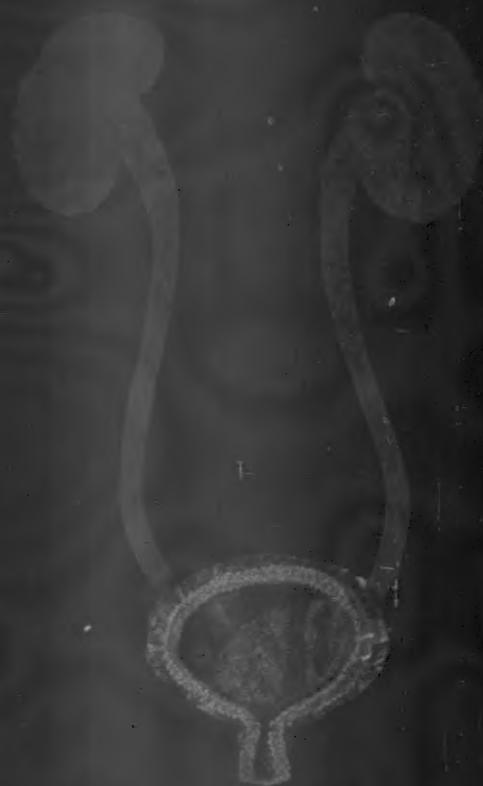
1. Frohman, I. P., Tranquilizers in General Practice and Clinical Evaluation of Deserpidine, an Alkaloid of Rauwolfia Canescens, M. Ann., District of Columbia, 27:641, Dec., 1958.
2. Billow, B. W., et al., The Use of a New Rauwolfia Derivative, Deserpidine, in Mild Functional Disturbances and Office Psychiatry, New York J. Med., 59:1789, May, 1959.
3. Rawls, W. B., et al., Clinical Experience with Deserpidine in the Management of Hypertension and Anxiety Neurosis, New York J. Med., 59:1774, May, 1959.

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1. Peck, F.B., Jr., and Griffith, R.S.: Antibiotics Annual 1957-1958, Medical Encyclopedia, Inc., p. 1004. 2. Wright, W.W., and Welch, H.: Antibiotic Med. 5:139 (Feb.) 1958.

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REFERENCES: 1. Editorial: J.M.A. Georgia 46:433, 1957. 2. Colby, F. H.: Essential Urology, Baltimore, The Williams & Wilkins Co., 1953, p. 330.

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1. Moyer, J. H.: M. Clin. North America, 7: 1957, p. 405.
2. Soldner, H. M.: Illinois M. J. 109:2.
3. Charles, C. M.: Geriatrics 11:1.
4. Weil, L. L.: J. Florida Acad. 4:9 No. 3, 1954.
5. Kinney, J. J.: New Jersey 53:126, 1956.
6. Semmens, J. P.: Gynec. 9:586, 1957.
7. Conner, J. H., and Moyer, J. H.: GP 14:124, No. 3, 1956.
8. C. W., et al.: South. M. J. 49:1, 1956.
9. C. McL., and Bryans, C. L., Jr.: 45:46, 1956.
10. Motion picture by Army, Navy, Air Force: M.A. 160:755, 1956.

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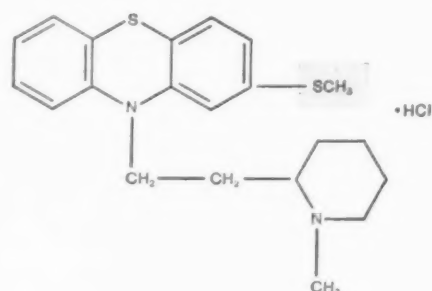
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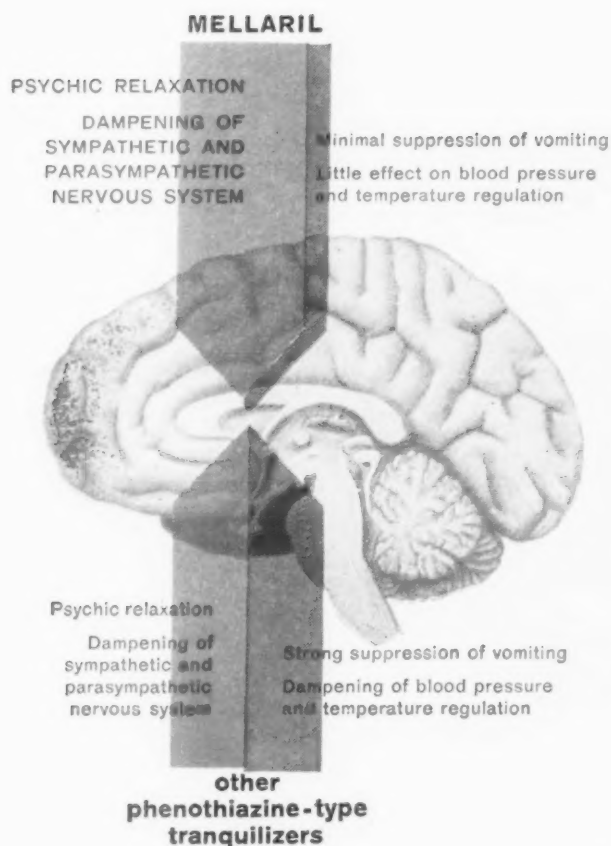
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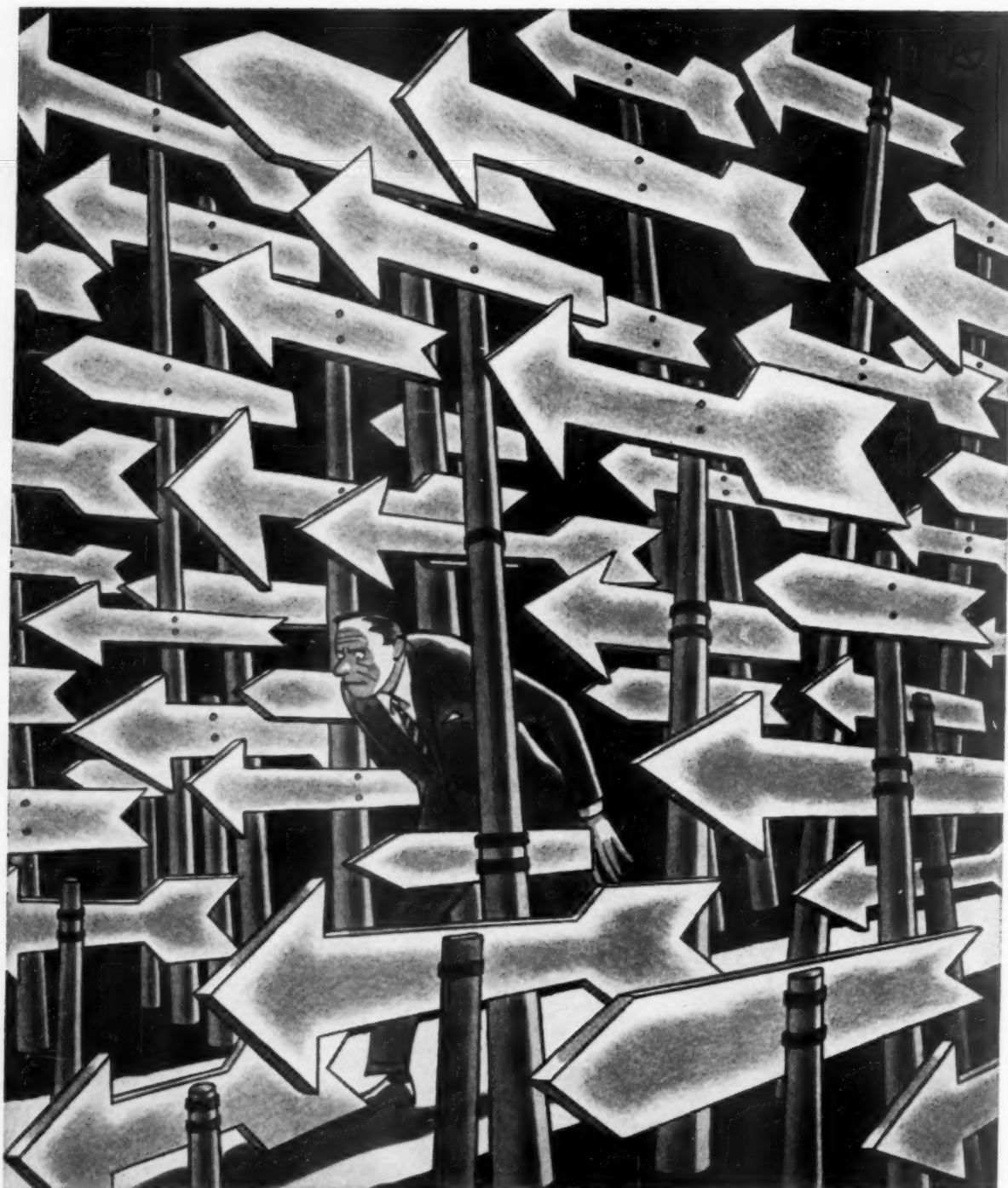
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*Ostfeld, A. M.: Scientific Exhibit, American Academy of General Practice, San Francisco, April 6-9, 1959.





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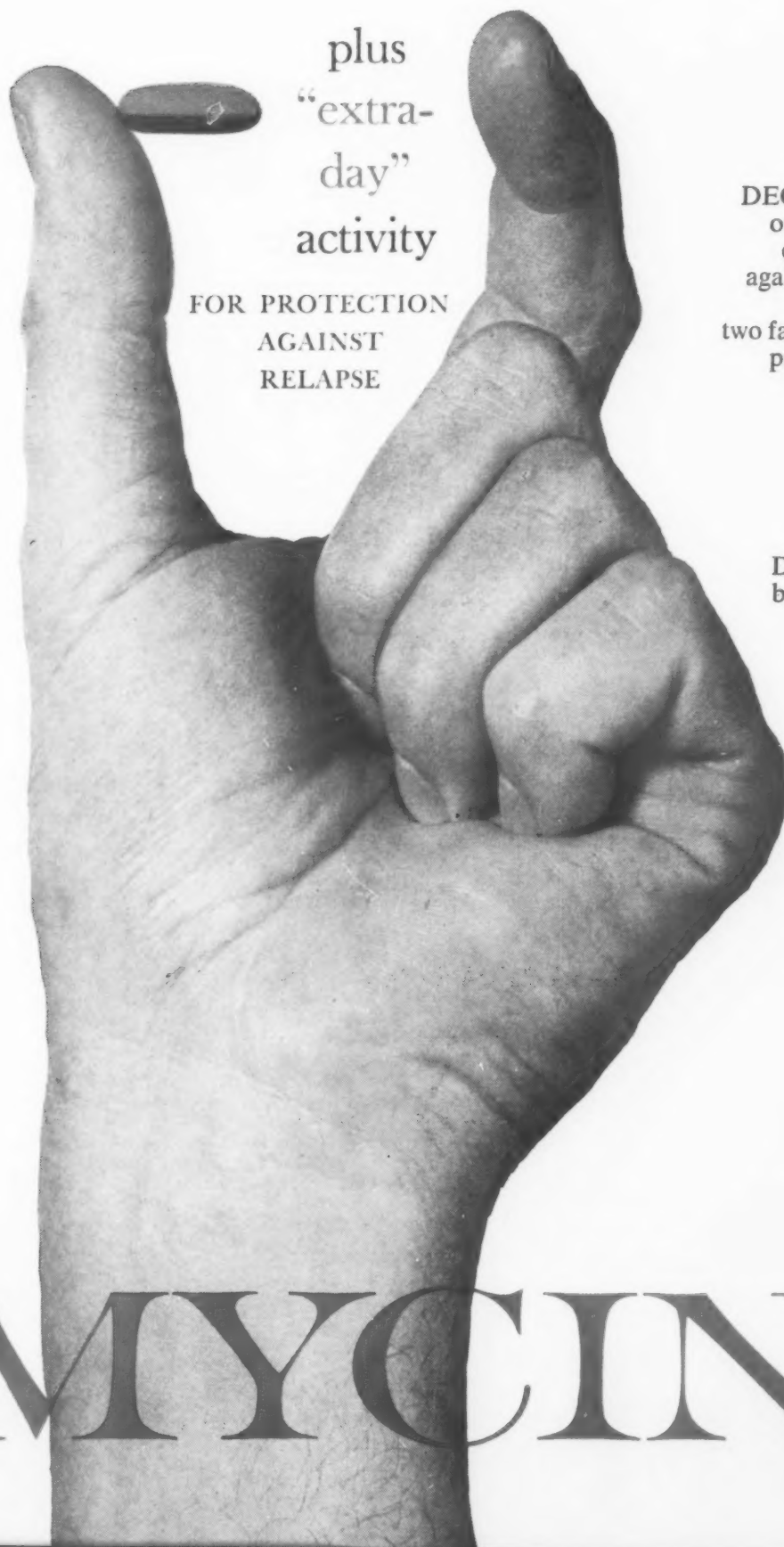
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*Hirsch, H. A., and Finland, M.:
New England J. Med. 260:1099
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Recommended dosage: First day — 1 teaspoonful (5 cc.) for each 18 lbs. body weight. Daily maintenance — ½ teaspoonful for each 18 lbs. body weight. Pediatric dosage should not exceed adult dosage. **Supplied:** 250 mg. per 5 cc., in 4-oz. bottles. Also available: MIDICEL[®] (sulfamethoxypyridazine, Parke-Davis), as quarter-scored tablets of 0.5 Gm., bottles of 24, 100, and 1,000. **Adult dosage:** Initial (first day) — 2 tablets (1 Gm.) for mild or moderate infections, or 4 tablets (2 Gm.) for severe infections. Maintenance — 1 tablet (0.5 Gm.) daily for mild or moderate infections, or 1 tablet (0.5 Gm.) to 3 tablets (1.5 Gm.) daily for severe infections, depending on severity of the infection and weight of the patient. **Children's dosage:** According to weight. See literature for details of dosage and administration.

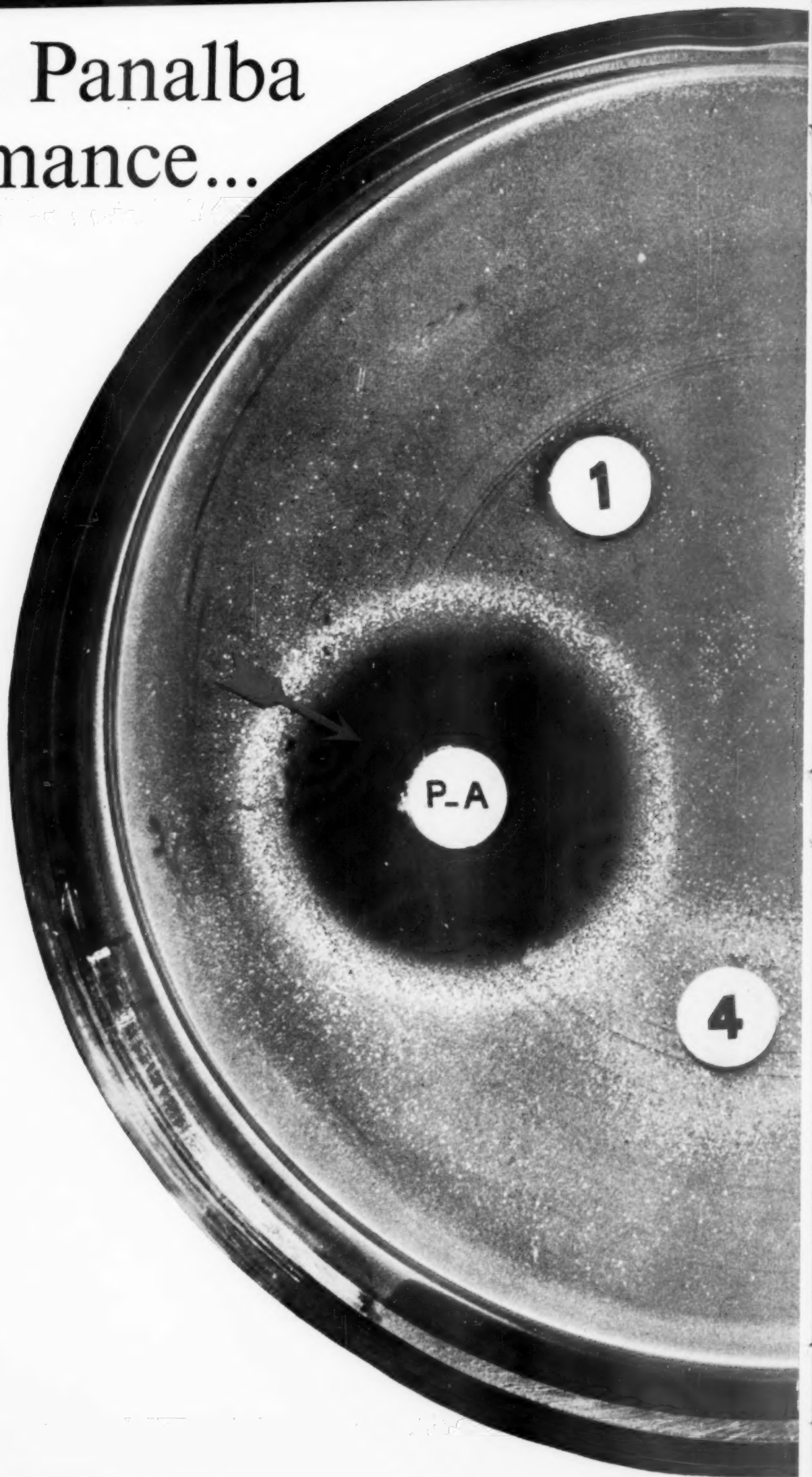


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This is Panalba
performance...



in bronchitis

... into a mixed culture of the four organisms commonly involved in bronchitis ... *Str. hemolyticus*, *D. pneumoniae*, *H. influenzae* and *Staph. aureus* (in this case a resistant strain) ... we introduce the five most frequently used antibiotics.

Twenty-four hours later (in this greatly enlarged photograph), note that only *one* of the five leading antibiotics has stopped *all* the organisms, including the resistant staph! This is Panalba.

In your next patient with bronchitis ... in *all* your patients with potentially-serious infections ... provide this extra protection with your prescription:

Dosage—1 or 2 capsules
3 or 4 times a day.
Supplied—Capsules containing
Panmycin phosphate equivalent
to 250 mg. tetracycline
hydrochloride, and 125 mg.
Albamycin as novobiocin
sodium, in bottles of 16 and 100.
Now available: new Panalba
Half-Strength Capsules in
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(Panmycin* Phosphate plus Albamycin*)

The broad-spectrum
antibiotic of
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Used in every type of practice
and clinical specialty today,
EQUANIL[®] (meprobamate, Wyeth)
has proved that anxiety and
tension *can* be controlled to
smooth the course of diagnosis,
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Anxiety is a fact of life. Anx-
iety is a fact of illness.



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in acute superficial thrombophlebitis

"A one-week course of therapy is generally sufficient to produce satisfactory resolution of the inflammatory process without recurrence."

Orbach, E. J.: J. Internat. Coll. Surgeons 31:165, 1959.

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"Patients who experienced major improvement had prompt and almost complete relief of pain and stiffness, which could be maintained on a small maintenance dose."

Graham, W.: Canad. M.A.J. 79:634, (Oct. 15) 1958.

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tablets • alka capsules

BUTAZOLIDIN® (brand of phenylbutazone): Red-coated tablets of 100 mg.

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ANTISPASMODIC ANTISECRETORY TRANQUILIZER

One preparation for multiple G.I. symptoms — ENARAX combines a new long-acting anticholinergic, oxyphen-cyclimine, with the proven antisecretory tranquilizer, ATARAX, to relieve pain, spasm, hyperacidity and tension associated with organic disturbances.

Two tablets daily for full-time relief — ENARAX successfully controlled symptoms in 94% of reported cases. In the majority of patients, two tablets daily in divided doses provided 24-hour control.

Relatively free of side effects — Side effects seldom consist of more than dryness of the mouth. Selective postganglionic action on the G.I. tract keeps adverse reactions to a minimum.⁴

SUMMARY OF CASES

Clinical Diagnosis — Peptic Ulcer — Gastritis — Gastroenteritis—Colitis—Functional Bowel Syndrome—Duodenitis Hiatus Hernia (Symptomatic) — Irritable Bowel Syndrome Pylorospasm — Cardiospasm — Biliary Tract Dysfunctions

| Clinical Results | Oxyphen-cyclimine ¹⁻³ | ENARAX ^{3,4} |
|-----------------------|----------------------------------|-----------------------|
| Effective | 491 (88%) | 223 (94%) |
| Failure | 68 (12%) | 15 (6%) |
| Total number of cases | 559 | 238 |

Each ENARAX tablet contains:

Oxyphen-cyclimine HCl10 mg.
Hydroxyzine (Atarax®).....25 mg.

Dosage: One-half to one tablet twice daily—preferably in the morning and before retiring. The maintenance dose should be adjusted according to therapeutic response. Use with caution in patients with prostatic hypertrophy and with ophthalmological supervision in glaucoma.

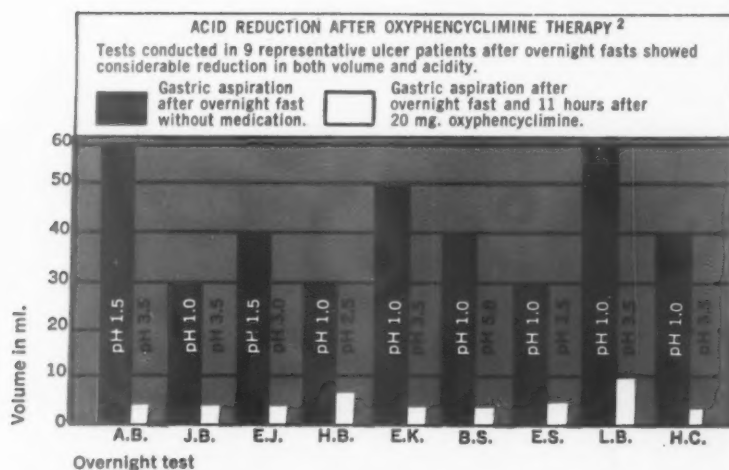
Supplied: In bottles of 60 black-and-white scored tablets.



ENARAX[®]

(oxyphenyclimine plus ATARAX[®])

A SENTRY FOR THE G.I. TRACT



References: 1. Stelgmann, F.: Study conducted at Cook County Hospital, Chicago, Illinois: In press. 2. Winkelstein, A.: Am. J. Gastroenterol. **32:66** (July) 1959. 3. Data in Roerig Medical Department files. 4. Leming, B. H., Jr.: Clin. Med. **6:423** (Mar.) 1959.



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REFLECTION ON
CORTICOTHERAPY:



The clinical aim, following immediate suppression of disease symptoms, is to maintain the patient symptom-free... with minimal side effects.

The logical course is to select the steroid with the best ratio of desired effects to undesired effects:

the corticosteroid that hits the disease, but spares the patient

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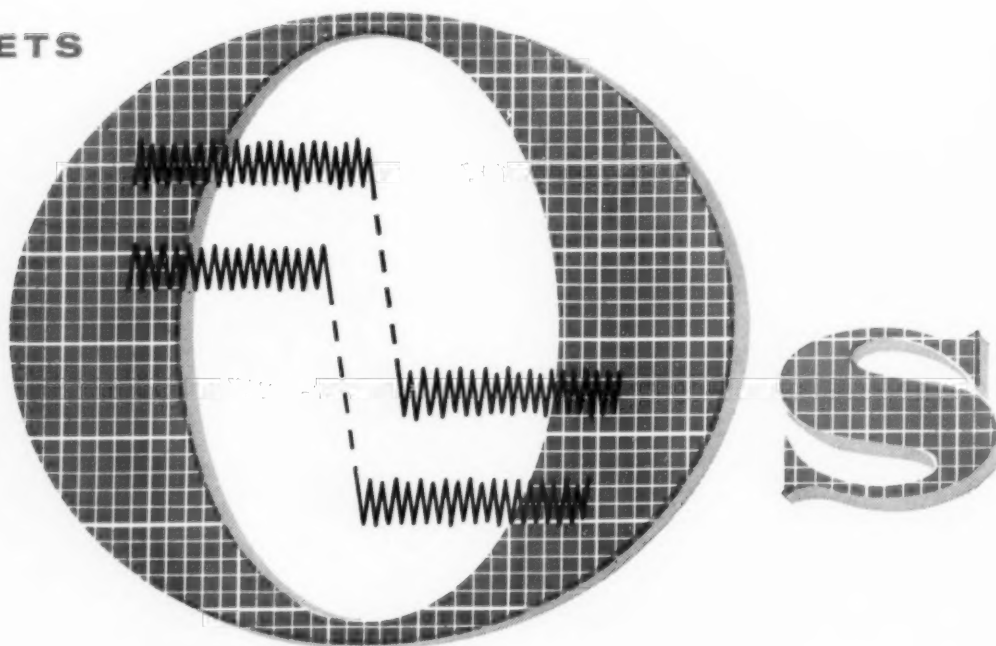
OSTENSIN is a new oral ganglionic-blocking agent of significance to every physician who treats hypertensive patients. The sympathetic blockade produced with OSTENSIN lowers systolic and diastolic blood pressure with predictable effectiveness and yet—because of minimal parasympathetic action—with fewer and less severe by-effects.^{1,2,3} OSTENSIN offers further advantages in oral administration, low dosage, smooth and prolonged antihypertensive action, prompt onset, no evidence of inherent toxicity, and rare drug tolerance.³

OSTENSIN, used with chlorothiazide or its derivatives, provides a superior new antihypertensive regimen^{1,2,3,4} with reduced dosage, by-effects further decreased, and maximal clinical benefits.

OSTENSIN is indicated in diastolic hypertension. (Diastolic hypertension is defined as "... the elevation of diastolic blood pressure to 90 mm. Hg or above."⁵)

COMPREHENSIVE LITERATURE SUPPLIED ON REQUEST

TABLETS



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OSTENSIN is the registered trademark for Trimethidinium Methosulfate, Wyeth.

effective antihypertensive regimen

BY-EFFECTS OF THREE OTHER GANGLIONIC-BLOCKING AGENTS^{6,7,8} COMPARED WITH THOSE OF OSTENSIN^{1,9}

| | Other Agents | OSTENSIN |
|----------------------|--------------------|-----------------|
| Constipation | 59-69% of patients | 5% of patients |
| Postural hypotension | 33-59% of patients | 37% of patients |
| Visual disturbances | 42-50% of patients | 34% of patients |
| Dry mouth | 38-41% of patients | 15% of patients |

"Of particular interest has been the virtual absence of constipation despite adequate blood pressure control. This finding suggests a lower risk of paralytic ileus. . . ."¹

Supplied: Tablets, scored, 20 and 40 mg., vials of 100.

1. Dunsmore, R.A., et al.: *Am. J. M. Sc.* 236:483 (Oct.) 1958. 2. Blaquier, P., et al.: *Univ. Michigan M. Bull.* 24:409 (Oct.) 1958. 3. Smirk, F.H.: Submitted for publication. 4. Janney, J.F.: Submitted for publication. 5. Council on Drugs, A.M.A.: *J.A.M.A.* 166:640 (Feb. 8) 1958. 6. Freis, E.D., and Wilson, I.M.: *Circulation* 13:856 (June) 1956. 7. Moyer, J.H., et al.: *A.M.A. Arch. Int. Med.* 98:187 (Aug.) 1956. 8. Moyer, J.H., et al.: *Am. Pract. & Dig. Treat.* 7:1765 (Nov.) 1956. 9. Dunsmore, R.A. In Tislow, R.F., et al.: *Scientific Exhibit. Presented at Annual Convention of A.M.A., San Francisco, June 23-27, 1958.*

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Ganglionic blockade with fewer and milder by-effects

How many patients today
complained about pain?



non-narcotic—pain relief equivalent
to that of codeine

well tolerated in both acute and
prolonged use

wide range of indications—general
practice and the specialties

analgesia plus anti-inflammatory
action

Supplied: Tablets, bottles of 48. Each tablet
contains 75 mg. of ethoheptazine citrate and
325 mg. (5 grains) of acetylsalicylic acid.

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Zactirin[®]

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the oral antidiabetic most likely to succeed



The superior effectiveness of DIABINESE increases the chance of success of oral therapy in your diabetic patients. Moreover, in properly regulated dosage, DIABINESE is free from significant incidence of serious side effects. Incidentally, your patients will appreciate the economy possible (savings up to 50%) when DIABINESE is the oral therapy selected.

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brand of chlorpropamide

economical once-a-day dosage



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when replacement or reduction of insulin is desirable...

"Patient B. G. . . . , a 64-year-old white male with diabetes of 5 years' duration regulated by 64 units of NPH and regular insulin...The effective replacement of so large a dose of insulin by so small a dose of sulfonylurea [250 mg. DIABINESE/day] is striking."

Beaser, S. B.: Ann. New York Acad. Sc. 74:701, 1959.



when other oral therapy has failed...

"Eleven diabetic patients who responded poorly to tolbutamide were treated with chlorpropamide. All responded better to chlorpropamide at considerably lower daily dosages in most cases."

Knauff, R. E.; Fajans, S. S.; Ramirez, E., and Conn, J. W.:
Ann. New York Acad. Sc. 74:603, 1959.



when dietary control proves impractical...

"Six patients were selected for a trial with chlorpropamide. These were all persons who had stable diabetes of the adult type and who could not be controlled by dietary management alone..."

"It can be seen that in all cases satisfactory postprandial control of the patient was obtained with chlorpropamide in varying doses."

Radding, R. S.: Texas J. Med. 55:110, 1959.

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economical once-a-day dosage

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TABLET MAALOX: 0.4 Gram (equivalent to one teaspoonful), Bottles of 100.

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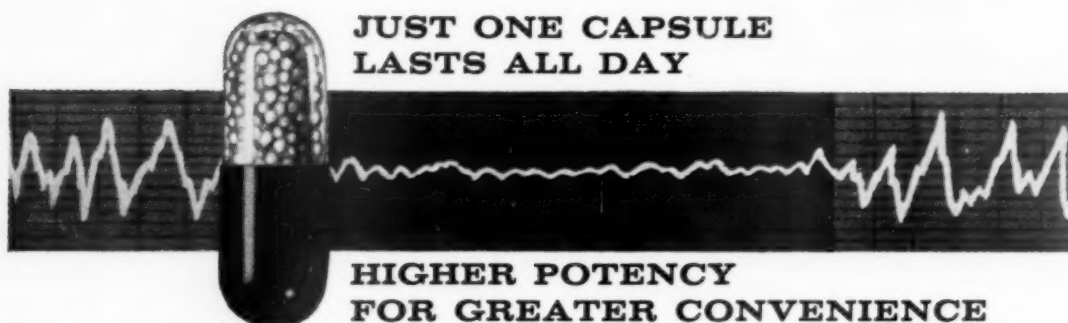
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HIGHER POTENCY
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- relieves *both* mental and muscular tension without causing depression
- does not impair mental efficiency, motor control, or normal behavior

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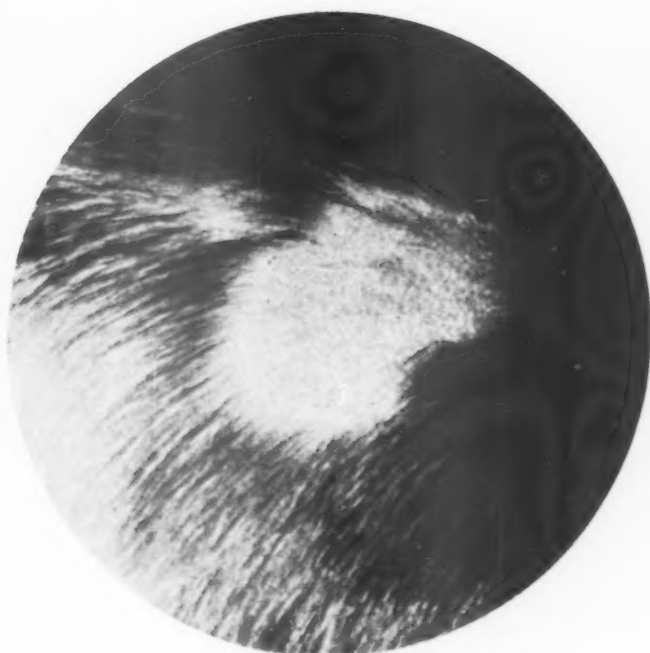
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Both potencies in bottles of 30.

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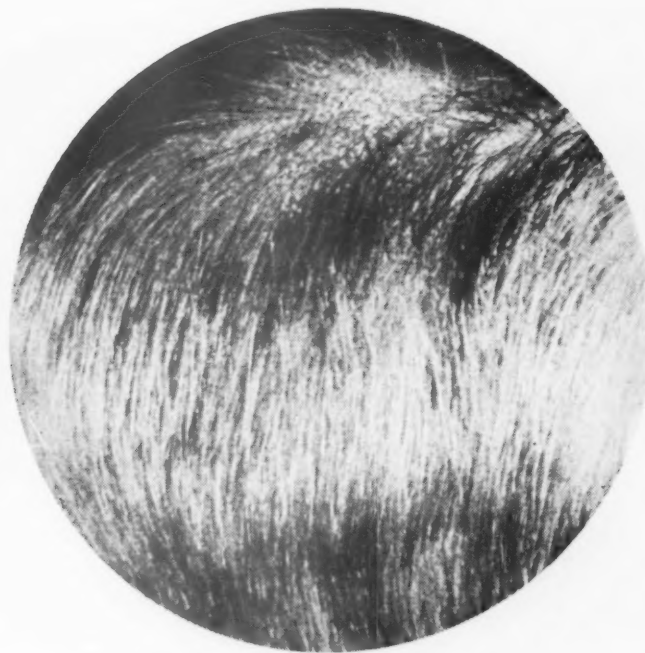
clears the tineas
from head to toe—
orally



In tinea capitis



Before FULVICIN: Tinea capitis (*Microsporum audouini*) in a 7-year-old boy.



After FULVICIN: Normal, new hair growth after 6 weeks of oral therapy.

Photos courtesy of M. M. Nierman, M.D., Calumet City, Ill.

Lesions clear, cultures become negative in

tinea corporis: 4 to 5 weeks¹

tinea cruris: 4 to 6 weeks¹

onychomycosis: 4 to 6 months¹

tinea pedis: 6 to 8 weeks¹

first oral fungistat to penetrate keratin from the inside... acts to check invading ring-worm fungi (*Microsporum*, *Trichophyton*, *Epidermophyton*)... usually well tolerated, side effects rare in therapeutic doses.

For complete information about dosage, indications and precautions consult Schering Statement of Directions.

Packaging: FULVICIN Tablets, 250 mg., bottles of 30 and 100.

1. Robinson, H. M., Jr., et al.: Griseofulvin, Clinical and Experimental Studies, A.M.A. Arch. Dermat., in press.

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EVEN IN "SEEMINGLY HOPELESS CASES" INVOLVING "HOSPITAL STAPH"...

"It would appear, therefore, that from this limited experience with 17 desperately ill patients, parenteral novobiocin [Albamycin] is therapeutically effective and offers a reasonable expectation of a favorable response even in seemingly hopeless cases."

Garry, M. W.: *Am. J. M. Sc.* 236:330 (Sept.) 1958.

"Staphylococcal sepsis, particularly as it appears within the hospital environment, continues to represent a serious and difficult therapeutic problem. . . . It would appear that novobiocin [Albamycin], like other broad-spectrum antimicro-

bial agents, will be of clinical value in a certain number of staphylococcal infections."

Colville, J. M.; Gale, H. H.; Cox, F., and Quinn, E. L.: *Antibiotics Annual 1957-1958*, p. 920.

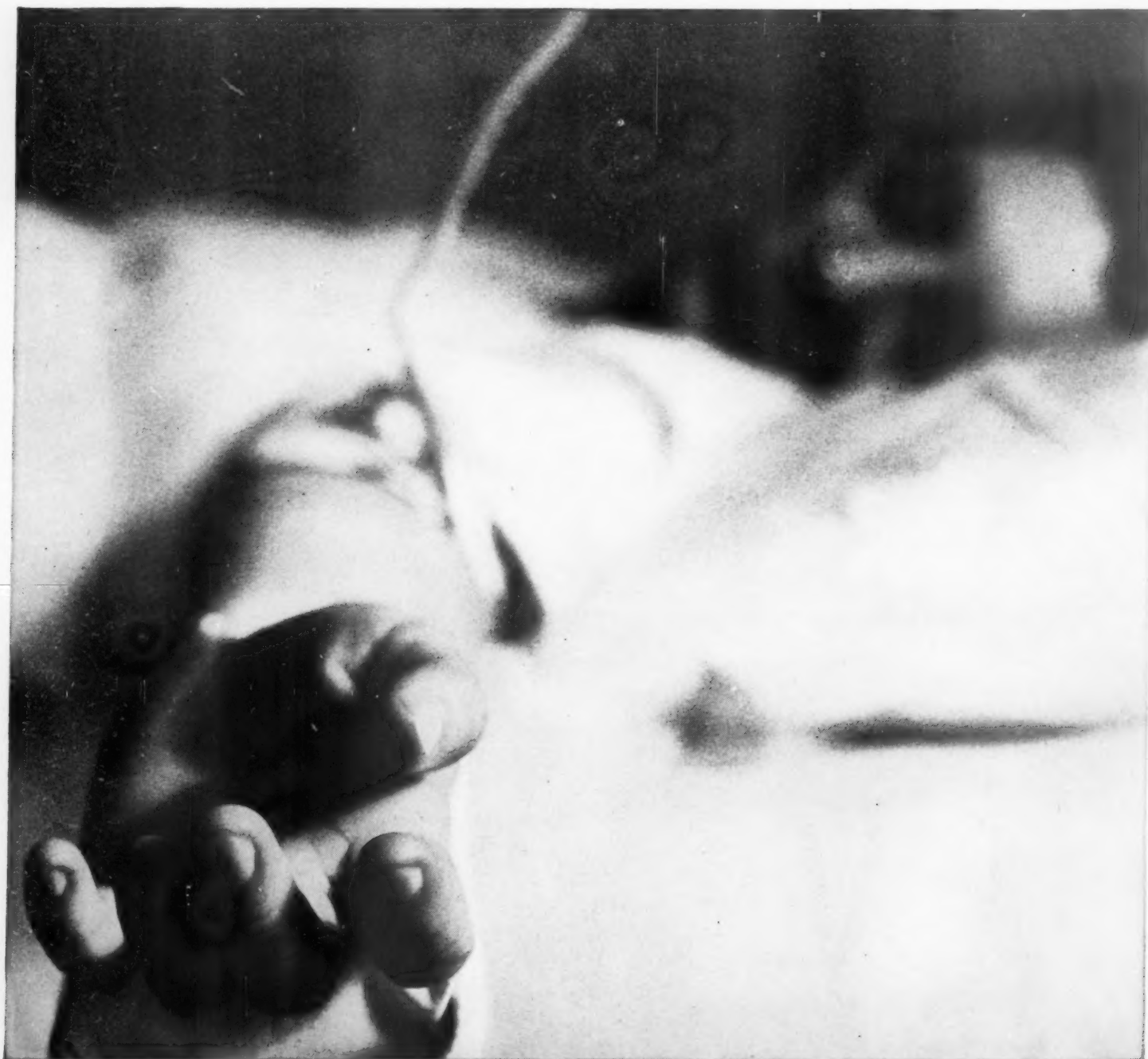
The use of Albamycin has not been accompanied by systemic toxicity — renal, hepatic, or hematopoietic. Side effects (such as skin rash) have been minor in nature, and those that do occur are easily managed.¹⁻³

1. Garry, M. W., *op. cit.* 2. Editorial, *New England J. Med.* 261:152 (July 16) 1959. 3. Nunn, D. B., and Parker, E. F.: *Am. Surgeon* 24:361 (May) 1958.

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ALBAMYCIN*



*TRADEMARK, REG. U. S. PAT. OFF. — THE UPJOHN BRAND OF CRYSTALLINE NOVOBIOCIN SODIUM


PROVEN EFFECTIVE FOR THE TENSE AND NERVOUS PATIENT



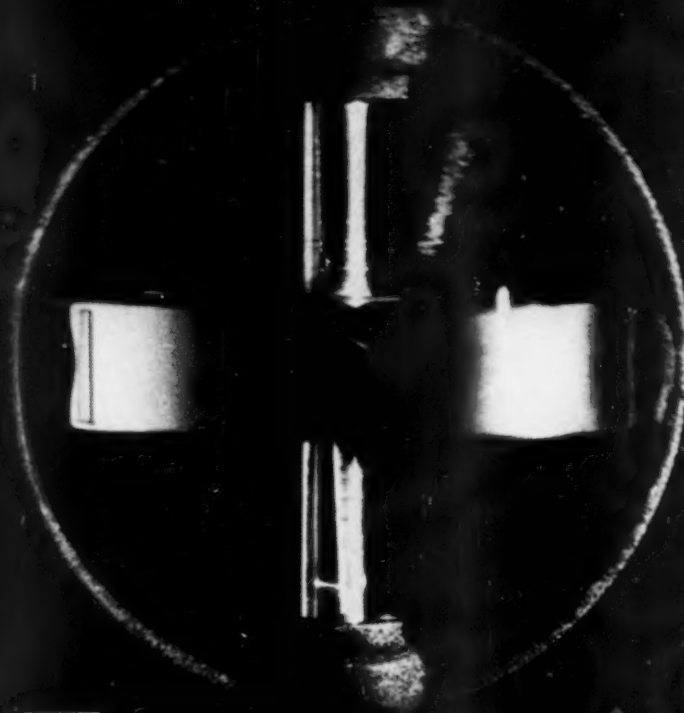
“There is perhaps no other drug introduced in recent years which has had such a broad spectrum of clinical application as has meprobamate.* As a tranquilizer, without an autonomic component in its action, and with a minimum of side effects, meprobamate has met a clinical need in anxiety states and many organic diseases with a tension component.”

Krantz, J. C., Jr.: The restless patient — A psychologic and pharmacologic viewpoint.
Current M. Digest
25:68, Feb. 1958.

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 WALLACE LABORATORIES, New Brunswick, N. J.

minimal disturbance
of the patient's chemical and psychic balance...



*still unsurpassed
for total
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benefits*

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Substantiated by published reports of leading clinicians:

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of allergic
and
inflammatory symptoms¹⁻²⁰
- minimal disturbance
of the patient's
chemical and psychic
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Triamcinolone LEDERLE

At anti-inflammatory and antiallergic levels ARISTOCORT means:

- freedom from salt and water retention
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- euphoria and depression rare
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Indications: rheumatoid arthritis; arthritis; respiratory allergies; allergic and inflammatory dermatoses; disseminated lupus erythematosus; nephrotic syndrome; lymphomas and leukemias.
Precautions: With ARISTOCORT all traditional precautions to corticosteroid therapy should be observed. Dosage should always be carefully adjusted to the smallest amount which will suppress symptoms. After patients have been on steroids for prolonged periods, discontinuance must be carried out gradually.

Supplied: Scored tablets of 1 mg. (yellow); 2 mg. (pink); 4 mg. (white); 16 mg. (white).
Diacetate Parenteral (for intra-articular and intrasynovial injection). Vials of 5 cc. (25 mg./cc.).

References: 1. Feinberg, S.M., Feinberg, A.R., and Fisherman, E.W.: *J.A.M.A.* 167:58 (May 3) 1958. 2. Epstein, J.I. and Sherwood, H.: *Connecticut Med.* 22:822 (Dec.) 1958. 3. Friedlaender, S. and Friedlaender, A.S.: *Antibiotic Med. & Clin. Ther.* 5:315 (May) 1958. 4. Segal, M.S. and Duvenci, J.: *Bull. Tufts North East M. Center* 4:71 (April-June) 1958. 5. Segal, M.S.: Report to the A.M.A. Council on Drugs, *J.A.M.A.* 169:1063 (March 7) 1958. 6. Sherwood, H. and Cooke, R.A.: *J. Allergy* 28:97 (Mar.) 1958. 7. Duke, C.J. and Oviedo, R.: *Antibiotic Med. & Clin. Ther.* 5:710 (Dec.) 1958. 8. McGavack, T.H.: *Clin. Med.* (June) 1958. 9. Freyberg, R.H.; Berntsen, C.A., and Hellman, L.: *Arthritis and Rheumatism* 1:215 (June) 1958. 10. Hartung, E.F.: *J.A.M.A.* 167:973 (June 21) 1958. 11. Hartung, E.F.: *J. Florida Acad. Gen. Pract.* 8:18, 1958. 12. Zuckner, J.; Ramsey, R.H.; Caciolo, C., and Gantner, G.E.: *Ann. Rheum. Dis.* 17:398 (Dec.) 1958. 13. Appel, B.; Tye, M.J., and Leibsohn, E.: *Antibiotic Med. & Clin. Ther.* 5:716 (Dec.) 1958. 14. Kalz, F.: *Canad. M.A.J.* 79:400 (Sept.) 1958. 15. Mullins, J.F., and Wilson, C.J.: *Texas State J. Med.* 54:648 (Sept.) 1958. 16. Shelley, W.B.; Harum, J.S., and Pillsbury, D.M.: *J.A.M.A.* 167:959 (June 21) 1958. 17. DuBois, E.F.: *J.A.M.A.* 167:1590 (July 26) 1958. 18. McGavack, T.H.; Kao, K.T.; Leake, D.A.; Bauer, H.G., and Berger, H.E.: *Am. J. Med. Sc.* 236:720 (Dec.) 1958. 19. Council on Drugs: *J.A.M.A.* 169:257 (Jan. 17) 1959. 20. Rein, C.R.; Fleischmajer, R., and Rosenthal, A.R.: *J.A.M.A.* 165:1821 (Dec. 7) 1957.

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SYMPOSIUM REPORT:

ALTAFUR in antibiotic-resistant staphylococcal infections

ALTAFUR proved superior to any other single agent against staphylococcal infections encountered in the pediatric section of a general hospital. Introduced during an epidemic of severe staphylococcal pneumonia and bronchiolitis in younger children, ALTAFUR was employed in treating a total of 59 infants or juvenile patients, most of whom had upper or lower respiratory tract involvement. Almost all had been given antibiotics without effect; 34 were judged severely or critically ill. Cures were obtained in 54 of these patients after a 3 to 10 day course of ALTAFUR. There was only one failure (results were inconclusive in the remaining four cases). Mixed infections with *Pneumococcus* or *Streptococcus* sp. also responded readily.

ALTAFUR was administered orally in varying dosage: the optimal dose is believed to be about 22 mg./Kg. daily.

Side effects were minimal in these patients, being limited to gastric intolerance in a few cases, usually controllable by giving the drug with or after meals. Laboratory studies performed before and after ALTAFUR treatment revealed no adverse influence on renal, hepatic or hematopoietic function, nor other signs of toxicity.

In vitro, staphylococci isolated in this series proved uniformly susceptible to ALTAFUR, whereas many strains were resistant to a variety of antibiotics. With ALTAFUR as with all nitrofurans, the lack of development of significant bacterial resistance is considered a major advantage over other antimicrobials.

Lysaught, J. N., and Cleaver, W.: Paper presented at the Symposium on Antibacterial Therapy, Michigan and Wayne County Academies of General Practice, Detroit, Sept. 12, 1959 (published Nov., 1959)

bright new star
in the antibacterial firmament

ALTAFURTM

brand of furaltadone

the first nitrofurantoin effective orally
in systemic bacterial infections

- Antimicrobial range encompasses the majority of common infections seen in everyday office practice and in the hospital
- Decisive bactericidal action against staphylococci, streptococci, pneumococci, coliforms
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- Development of significant bacterial resistance has not been encountered
- Low order of side effects
- Does not destroy normal intestinal flora nor encourage monilial overgrowth (little or no fecal excretion)

Tablets of 50 mg. (pediatric) and 250 mg. (adult)

Average adult dose: 250 mg. four times a day, with food or milk

Pediatric dosage: 22-25 mg./Kg. (10-11.5 mg./lb. body weight daily in 4 divided doses)

CAUTION: The ingestion of alcohol in any form, medicinal or beverage, should be avoided during Altafur therapy.

NITROFURANS—a *unique* class of antimicrobials
EATON LABORATORIES, NORWICH, NEW YORK

VISTARIL[®]

hydroxyzine
pamoate

ORAL SUSPENSION

restores tranquility: relieves pruritus

Simultaneously, Vistaril releases tension and relieves itching. Vistaril tranquilizes patients gently without impairing mental alertness.

In 1958, the A.M.A. Council on Drugs designated Vistaril as a psychotherapeutic antihistamine.

A Professional Information
Booklet on Vistaril available
on request.



Suggested oral dosage — adjust according to response:

Children over 6, 50-100 mg. daily in divided doses.

Children under 6, 50 mg. daily in divided doses.

Vistaril is supplied as **Oral Suspension** — 25 mg. per teaspoonful (5 cc.), 1 pint bottles.

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Parenteral Solution (as the HCl) — 25 mg. per cc.; 10-cc. vials and 2-cc. Steraject[®] Cartridges.

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ISORDIL



a new
coronary vasodilator
of
unprecedented effectiveness
for
angina pectoris



- 1 rapid onset
- 2 prolonged action
- 3 consistent effect
- 4 unusual safety

ISORDIL significantly reduces the number, duration, and severity of anginal attacks, often when other long-acting coronary vasodilators fail. Exercise tolerance is increased, pain decreased, and the requirements for nitroglycerin either drastically curtailed or eliminated.

ISORDIL acts rapidly in comparison with other prophylactic agents, and patients usually experience benefits within 15 to 30 minutes. The effects of a single dose of ISORDIL persist for 4 to 5 hours. Thus, for most patients, convenient q.i.d. administration is highly satisfactory.

The only side effect observed has been transitory, easily controlled headache, normally considered an expression of effective pharmacodynamic activity.¹ The toxicity of ISORDIL is extremely low, approximately 50 times the therapeutic dose being required to produce toxic symptoms.

Sherber,² summarizing his experience with ISORDIL, states it is "the most effective medication for the treatment of coronary insufficiency available today."

ISORDIL



IVES-CAMERON COMPANY • New York 16, New York

Clinical and Laboratory Data Confirm Superiority

Succeeds where others fail:

Among 48 patients³ previously treated with other coronary vasodilators, chiefly pentaerythritol tetranitrate, ISORDIL was demonstrably superior in 37, equivalent in 9, and inferior in 2. Response of patients treated in all studies⁴ was 85% good, 7% fair, and 8% poor.

Markedly reduces number of anginal attacks:

Albert⁵ found that of 29 patients receiving ISORDIL, 25 responded well, 1 moderately well, and 1 not at all. Effectiveness could not be judged in 2 patients. For those who responded well, the frequency of anginal attacks was quickly reduced from a daily average of 5 to 1.2. Continued use of ISORDIL further reduced the frequency of attacks.

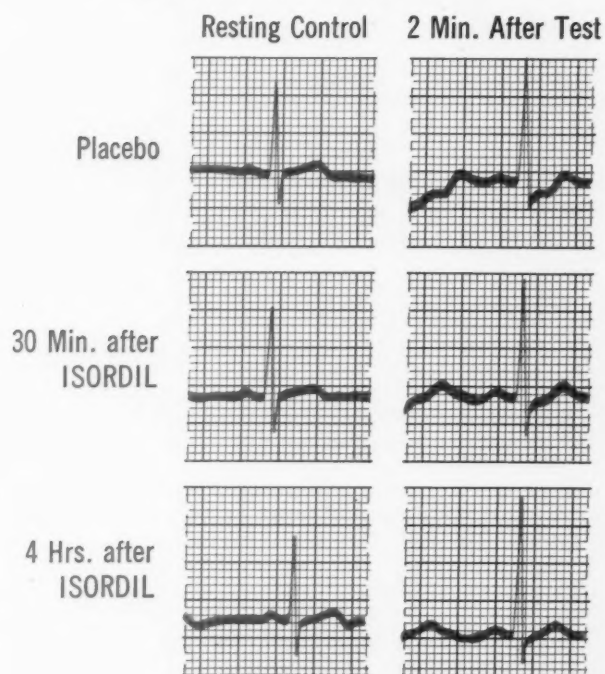
Increases tolerance to exercise and stress:

Electrocardiographic response following the Master two-step test has clearly established a more favorable balance between oxygen supply and demand to the myocardium with ISORDIL therapy. Eight of 10 patients administered ISORDIL in studies by Russek⁶ showed considerably less abnormality in the post-exercise electrocardiogram than before treatment.

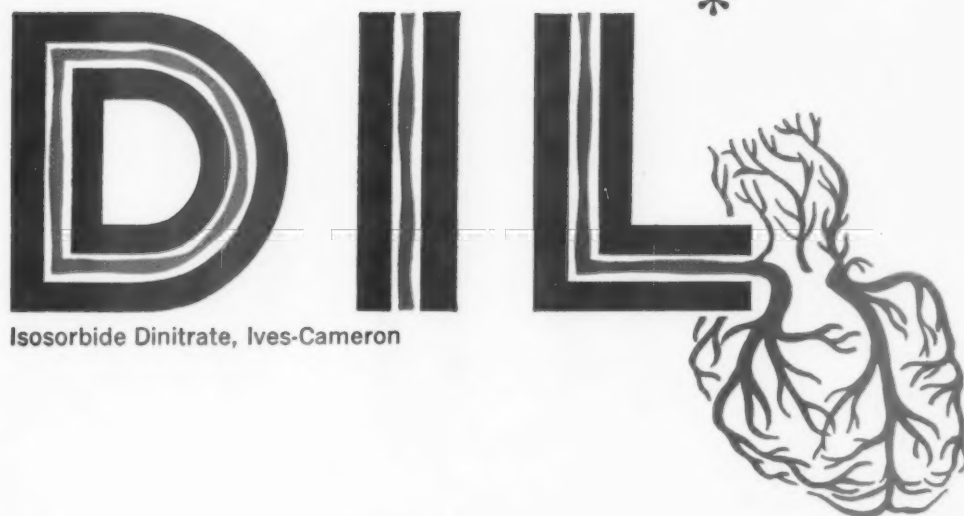
Rapid onset and prolonged action a function of solubility and metabolism:

Pharmacologic studies indicate that the rapid onset and prolonged action shown by ISORDIL are related to its high solubility and low rate of metabolism.⁷ Incubation with liver slices suggest rapid absorption and delayed inactivation by the liver.

Master Test Responses (Lead V₄) in a 58-Year-Old Male with Angina Pectoris⁶



unprecedented effectiveness
in angina pectoris



Isosorbide Dinitrate, Ives-Cameron

*Trademark

- NEW—for more effective control of angina pectoris
- Reduces number, duration, and severity of anginal attacks

***"Isordil is a new and effective agent for
therapy of angina pectoris."—Russek⁶***

Composition: Each white, scored tablet of ISORDIL (Isosorbide Dinitrate) contains 10 mg. of 1,4,3,6-dianhydro-sorbitol-2,5-dinitrate.

Action: Following oral administration of ISORDIL, the effects of coronary vasodilatation are apparent within 15 to 30 minutes and persist for 4 to 5 hours.

Indications: ISORDIL is indicated for the therapeutic and prophylactic management of angina pectoris and coronary insufficiency. It is often useful in patients only partially responsive to other long-acting coronary vasodilators.

Dosage: ISORDIL is administered orally. Average dose is one tablet (10 mg.) taken one half hour before meals and at bedtime. Individualization of dosage may be necessary for optimum therapeutic effect; dosage may vary from 5 mg. to 20 mg. q.i.d.

Side Effects: Side effects are few, infrequent, and mild. Transitory headache, common to effective nitrate or nitrite therapy, has occurred. This usually responds to administration of acetylsalicylic acid, and disappears with continued therapy. When headache is persistent, reduction in dosage may be required.

Caution: ISORDIL should be given with caution in patients with glaucoma.

Supplied: Bottles of 100.

References: 1. Riseman, J.E.F., et al.: *Circulation* 17:22-39 (Jan.) 1958. 2. Sherber, D.A.: Personal Communication (Oct., 1959). 3. Case Reports on File, Ives-Cameron Company (1958-1959). 4. Summary of Case Reports on File, Ives-Cameron Company (1958-1959). 5. Albert, A.: Personal Communication (Oct., 1959). 6. Russek, H.I.: Personal Communication (Oct., 1959). 7. Harris, E., et al.: Personal Communication (Oct., 1959).

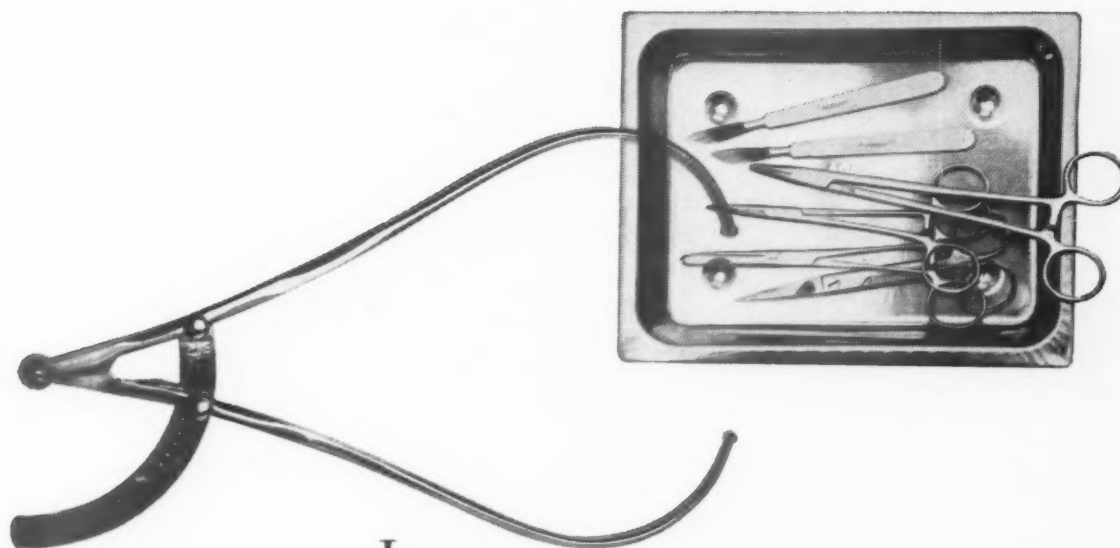
ISORDIL



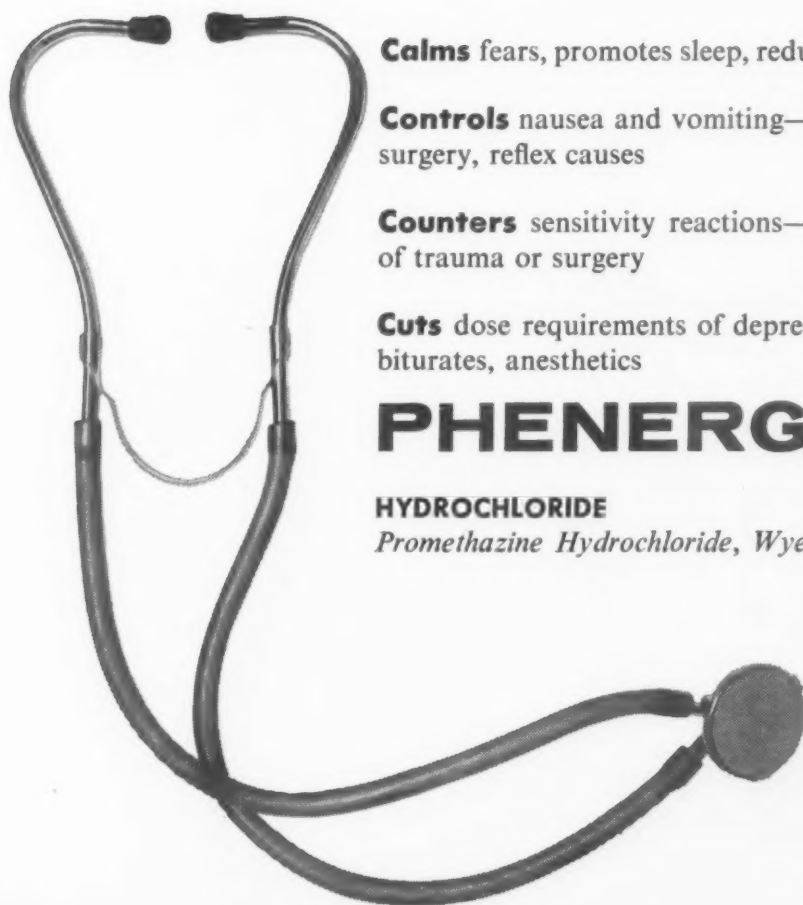
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Calms fears, promotes sleep, reduces postoperative excitement

Controls nausea and vomiting—motion sickness, pregnancy, surgery, reflex causes

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—curbs the desire for food, at the same time combats depression that may accompany dieting.
The result is less interest in eating, more interest in other activities.

SYNDROX TABLETS 5 mg.
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for better management of hypertension

moderil[®]
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*a purified alkaloid of rauwolfia...
 lessens the frequency and/or severity
 of these reserpine side effects:*

mental depression • bradycardia • sedation
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useful *alone* for gradual, sustained
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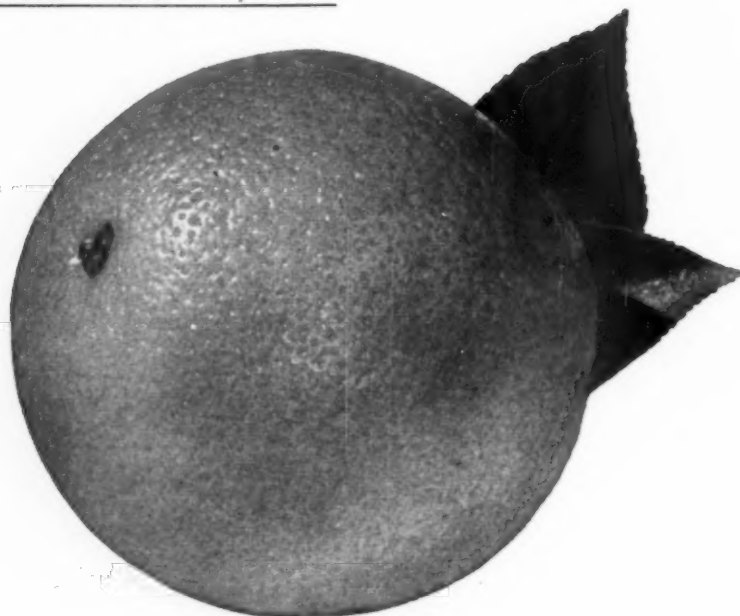


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even
if your
patient
is a
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he'll be under way again soon, once he's on

PARAFON[®]

(PARAFLEX[®] + TYLENOL[®])

for muscle relaxation plus analgesia

and in arthritis

PARAFON[®]
with Prednisolone

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prescribe PARAFON in low back pain—sprains—
strains—rheumatic pains

Each PARAFON tablet contains:

PARAFLEX[®] Chlorzoxazone† 125 mg.

Specific for skeletal muscle spasm

TYLENOL[®] Acetaminophen 300 mg.

The analgesic preferred in musculoskeletal pain

Dosage: Two tablets t.i.d. or q.i.d.

Supplied: Tablets, scored, pink, bottles of 50.

Each PARAFON WITH PREDNISOLONE tablet contains:

PARAFLEX[®] Chlorzoxazone† 125 mg., TYLENOL[®]

Acetaminophen 300 mg., and prednisolone 1.0 mg.

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Supplied: Tablets, scored, buff colored, bottles of 36.

Precautions: The precautions and contraindications
that apply to all steroids should be kept in mind
when prescribing PARAFON WITH PREDNISOLONE.

*sailor

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254A89

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NEW
MYOGESIC^x**



RELATM EASES STRAINS
SPRAINS & LOW
BACK PAINS...!

CARISOPRODOL

RELA—a new myogestic for better relaxant *and* analgesic therapy—more adept management of spasm and pain in strains, sprains and low back pains.

RELA—though a single drug—is a true myogestic and works rapidly to achieve three desired effects...



Rela relaxes acute muscle spasm

Relief of muscle spasm (96% excellent to good effectiveness)¹

Rela provides a unique quality of persistent pain relief through its relaxant and analgesic actions

"Relief from pain was usually rapid and sometimes dramatic"¹

Rela, through relaxation and analgesia, assures daytime ease and nighttime rest

"... A number of patients reported freedom from insomnia which they attributed to freedom from pain."¹

indications: RELA is most beneficial in those conditions of the musculoskeletal system manifesting pain, stiffness and spasm.

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supply: RELA is available as 350 mg., pink, coated tablets in bottles of 30.

1. Kuge, T.: To be published.

H-227

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(sulfipyrazone GEIGY)

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By significantly increasing renal excretion of urate and thus lowering plasma uric acid, the new highly potent uricosuric agent ANTURAN strikes directly at the basic metabolic defect in gout.

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Clinically, ANTURAN:

- Prevents formation of new tophi
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- Relieves chronic pain
- Restores joint mobility

ANTURAN is not designed for the treatment of acute attacks for which BUTAZOLIDIN[®] is recommended. Detailed Information On Request

YU, T. F.; Burns, J. J., and Gutman, A. B. Arth. & Rheumat. 1:532, 1958.

ANTURANTM (sulfipyrazone GEIGY). Scored tablets of 100 mg. in bottles of 100.

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Fluphenazine dihydrochloride

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safely controls the "target symptoms" of
emotional stress with the smallest effective dosage
of any neuroleptic* agent (0.25 mg. b.i.d.)

virtually free from side effects at the
recommended dosage level

a significantly wider spectrum of "target symptoms"
is amenable to therapy

onset of action is rapid; duration of effect is prolonged

***Neuroleptic** "The term 'neuroleptic' implies a specific effect of a pharmacologic agent on the nervous system. It refers to a mode of action on affective tension that distinguishes this response from that to hypnotic drugs. The terms 'ataraxics' and 'tranquilizers' are descriptively impressive, but fail to convey what seems psychopharmacologically unique."¹

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"Fluphenazine (PERMITIL) the latest and most potent phenothiazine tranquilizer was administered from 3 to 20 months to 200 ambulatory and hospitalized patients representing a full spectrum of diagnostic classifications including psychosomatic disorders. Fractional doses of this drug rapidly produced improvement in 74% of these patients while causing a minimum of sedative, autonomic and endocrine effects which disappeared as treatment continued. . . . Patient acceptance of this compound was excellent because its prescription facilitated rather than interfered with the efficient performance of daily tasks. The physician who masters the art of fluphenazine use can treat a widened spectrum of target symptoms, safely and effectively."²



Now,
for the first time,
a phenothiazine
anti-anxiety agent

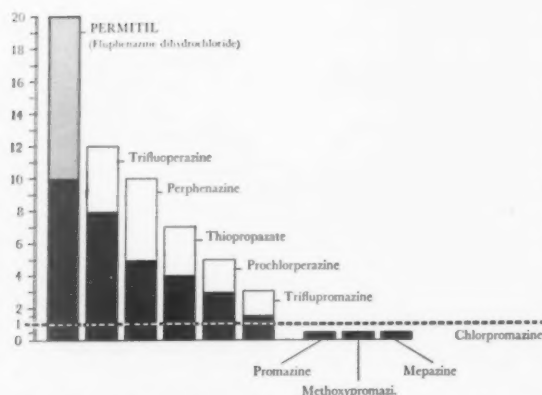
PERMITIL

designed specifically for
everyday office practice

A Factor to Consider in Phenothiazine Therapy

"The more potent the phenothiazine derivative the fewer the side effects it produces, because less of the chemical is needed to effect behavioral and therapeutic changes."²

The Relative Therapeutic Potency
of Various Phenothiazines



Adapted from Ayd, F. J., Jr.³

Clinical Results with PERMITIL—a Phenothiazine. In one study² covering a two-year period, PERMITIL was prescribed for 200 patients who were disabled primarily by anxiety and its somatic, emotional, mental and behavioral effects.

"After 3 months of fluphenazine (PERMITIL) therapy, 74% or 148 of the 200 patients evaluated were improved. . . . Thus the therapeutic effectiveness of fluphenazine (PERMITIL) is somewhat better than that of other potent tranquilizers."²

The relatively minor somatic reactions occurred in the early weeks of treatment with doses above 2 mg. daily. They seldom required other medication to counteract them and disappeared as the maintenance dose was established. At dosage levels under 3 mg. a day, extrapyramidal side effects were minimal. Results of extensive laboratory tests on 130 patients disclosed that "fluphenazine (PERMITIL) administered over 3 to 18 months had no deleterious effect on the blood, liver or kidney in these patients."²

The Importance of PERMITIL in Everyday Practice. "In contrast to other phenothiazines, it (PERMITIL) mitigates apathy, indifference, inertia and anxiety-induced fatigue. Thus, instead of impeding effective performance of daily tasks, it increases efficiency by facilitating psychic relaxation. Consequently, acceptance of this drug, especially by office patients, has been excellent."²

How to Prescribe PERMITIL. For most adults: One 0.25 mg. tablet b.i.d. (taken morning and afternoon). In refractory cases: Two 0.25 mg. tablets b.i.d. Total daily dosage in refractory cases should not exceed 2 mg., in divided doses. Dosage for children has not been established. *Complete information concerning the use of this drug is available on request.* Available as Tablets, 0.25 mg., bottles of 50 and 500.

References: 1. Freyhan, F. A.: Psychopharmacology Frontiers, Boston, Little, Brown and Co., 1959, p. 7. 2. Ayd, F. J., Jr.: Fluphenazine: its spectrum of therapeutic application and clinical results in psychiatric patients, Current Therapeutic Research, 1:41 (Oct. 15) 1959. 3. Ayd, F. J., Jr.: The current status of major tranquilizers, in press.

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ONE PITCH AND I STRUCK OUT...



Just an hour of pitching baseball with my son and I "threw my arm out." Painful? I couldn't even sleep that night!



Next day on the job I was a total loss — could hardly lift a slide rule!



I saw my doctor that night and got a prescription for some tablets.

Percodan® Demi & Percodan® Tablets *FOR PAIN*

Salts of Dihydrohydroxycodeinone and Homatropine, plus APC

ACTS FASTER — usually within 5-15 minutes. **LASTS LONGER** — usually 6 hours or more. **MORE THOROUGH RELIEF** — permits uninterrupted sleep through the night. **RARELY CONSTIPATES** — excellent for chronic or bedridden patients. **VERSATILE** — new "demi" strength permits dosage flexibility to meet each patient's specific needs. PERCODAN-DEMI provides the PERCODAN formula with one-half the amount of salts of dihydrohydroxycodeinone and homatropine.

AVERAGE ADULT DOSE: 1 tablet every 6 hours. May be habit-forming. Federal law permits oral prescription.

Each PERCODAN® Tablet contains 4.50 mg. dihydrohydroxycodeinone hydrochloride, 0.38 mg. dihydrohydroxycodeinone terephthalate, 0.38 mg. homatropine terephthalate, 224 mg. acetylsalicylic acid, 160 mg. phenacetin, and 32 mg. caffeine.

*U.S. PAT. 2,828,195

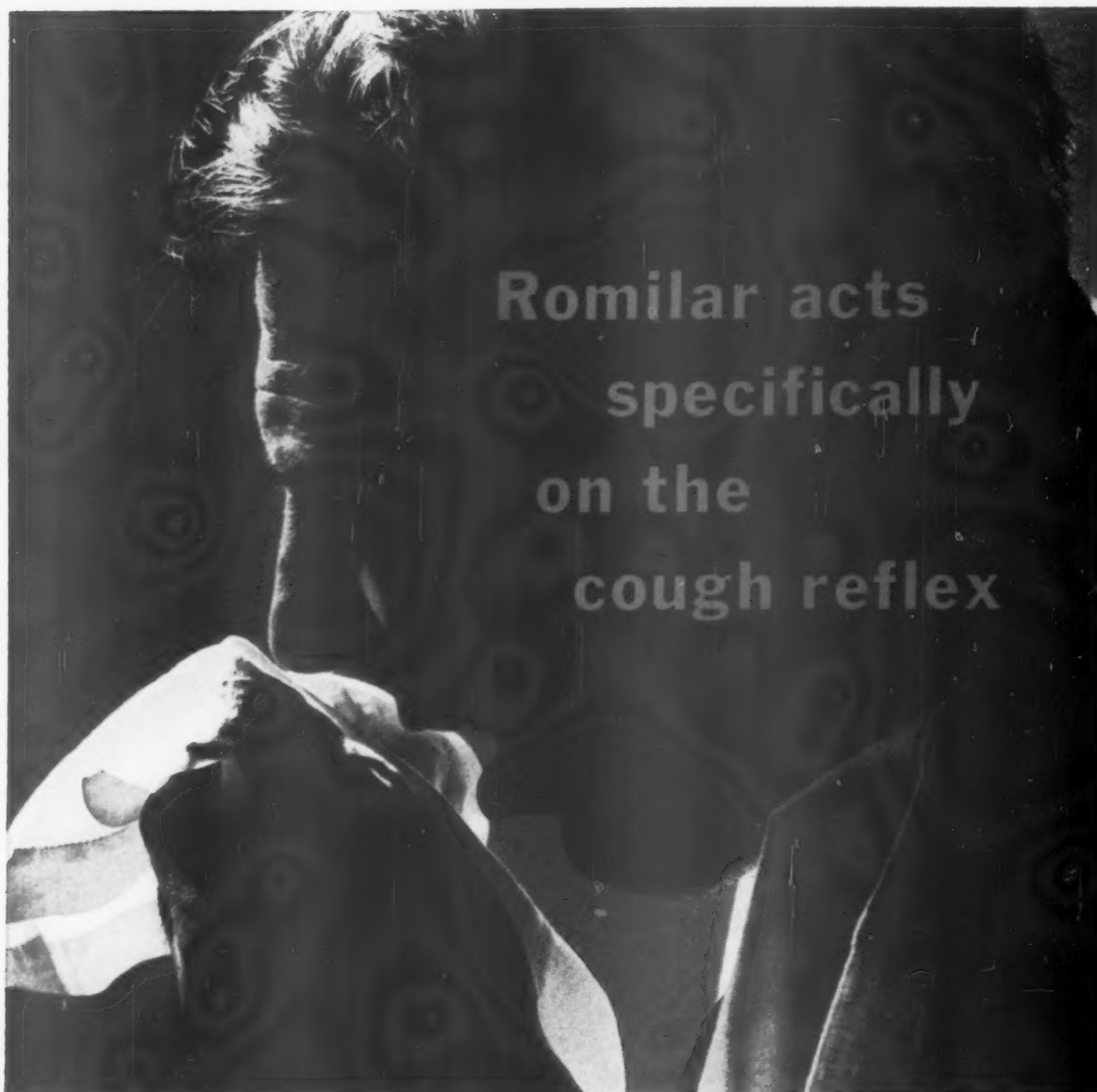


Amazing! The pain went away fast—in about 15 minutes. I slept like a baby. Finished my design for the new warehouse next day, and not a bit of trouble since!

AND THE PAIN WENT AWAY FAST

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Literature? Write
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When cough causes strain and trauma...in the elderly and debilitated; in patients with pulmonary and cardiac disease, hernia or rib fracture; in EENT, chest and abdominal surgery...with ROMILAR you can provide the antitussive potency of codeine with the safety of a placebo.¹⁻⁹ Cough control is prompt (15-30 minutes) and lasts for as long as six hours.¹

ROMILAR, the non-narcotic cough specific, has one pharmacologic action—cough suppression. There is no central depressant or analgesic effect; and there is no tolerance liability, no addictive potential.²⁻⁹

Bibliography: (1) N. Ralph, *Am. J. M. Sc.* 227:297, 1954. (2) H. A. Bickerman, in W. Modell, Ed., *Drugs of Choice 1958-1959*, St. Louis, The C. V. Mosby Company, p. 557. (3) H. A. Bickerman, E. German, B. M. Cohen and S. Itkin, *Am. J. M. Sc.* 234:191, 1957. (4) L. J. Cass, W. S. Frederik and J. B. Andosca, *Am. J. M. Sc.* 227:291, 1954. (5) L. J. Cass and W. S. Frederik, *J. Lab. & Clin. Med.* 48:879, 1956. (6) L. J. Cass and W. S. Frederik, *New England J. Med.* 249:132, 1953. (7) H. Isbell and H. F. Fraser, *J. Pharmacol. & Exper. Therap.* 107:524, 1953. (8) W. M. Benson, P. L. Steffo and L. O. Randall, *J. Pharmacol. & Exper. Therap.* 109:189, 1953. (9) *New and Nonofficial Drugs 1959*, Philadelphia, J. B. Lippincott Company, p. 326.

Supply: ROMILAR Syrup in bottles of 4 oz, 16 oz and 1 gal. ROMILAR Tablets in bottles of 20, 100 and 500. Romilar® Hydrobromide—brand of dextromethorphan hydrobromide.

ROCHE LABORATORIES • Division of Hoffmann-La Roche Inc • Nutley 10, N. J.

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*when every
complaint
reacts on
her stomach*

TO HELP HER G.I. TRACT—AND HER—REGAIN THEIR COMPOSURE

BUTIBEL[®]

antispasmodic-sedative

"quiets down" the queasy, spastic colon—through the gentle sedation of BUTISOL Sodium[®] butabarbital sodium 15 mg. and the relaxing action of *natural* extract of belladonna 15 mg. The central and peripheral effects of BUTIBEL are of approximately equal duration—no overlapping sedation or inadequate spasmolysis.

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“R Day”

*for the neuritis patient
can be tomorrow*

“R Day”—when pain is relieved—can come early for patients with inflammatory (non-traumatic) neuritis if treatment with Protamide is started promptly after onset.

Protamide is the therapy of choice for either early or delayed treatment, but early use assures greatest efficacy.

For example, in a 4-year study¹ and a 26-month study² a combined total of 374 neuritis patients treated with Protamide during the first week of symptoms responded as follows:

60% required only 1 or 2 daily injections for complete relief

96% experienced excellent or good results with 5 or less injections

Thus, the neuritis patient's first visit—especially an early one—affords the opportunity to speed his personal “R Day.”

Protamide is available at pharmacies and supply houses in boxes of ten 1.3 cc. ampuls. Intramuscularly only, one ampul daily.

PROTAMIDE®



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Sherman Laboratories
Detroit 11, Michigan



1. Lehrer, H. W., et al.: Northwest Med. 75:1249, 1955.

2. Smith, Richard T.: New York Med. 8:16, 1952.

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INTRAMUSCULAR IRON-DEXTRAN COMPLEX

corrects and prevents iron deficiency in blood and marrow

PEDIATRICS: "IMFERON has the advantage of safe and easy administration; treatment is completed in a few days and is not influenced by feeding problems."¹

OBSTETRICS: "...we have been able to raise hemoglobin levels of 7 or 8 Gm. to normal figures within a few weeks...."²

CHRONIC BLOOD LOSS: IMFERON "...is also to be preferred to blood transfusions for correcting the effects of chronic blood loss. The risk of transfusion reactions is avoided, as well as the dangers of contamination and sensitization. Besides improving the anemia, iron stores will be replenished...."³

GERIATRICS: A 66-year-old woman with recurrent gastrointestinal bleeding for over six years [two abdominal explorations, source undiscovered] "...has been maintained at a comfortable blood level for over nine months on intramuscular iron injection, with greatly reduced transfusion requirement."⁴

SUPPLIED: 2-cc. and 5-cc. ampuls; 10-cc. multiple-dose vials. There are 50 mg. of elemental iron per cc.

(1) Wallerstein, R. O., and Hoag, M. S.: J.A.M.A. 164:962 (June 29) 1957. (2) Eastman, N. J.: Current M. Dig. 25:55 (Jan.) 1958. (3) Koszewski, B. J., and Walsh, J. R.: Am. J. M. Sc. 235:523 (May) 1958. (4) McCurdy, P. R.; Rath, C. E., and Meerkrebs, G. E.: New England J. Med. 257:1147 (Dec. 12) 1957.

Clarín* can do this for your postcoronary patients



WITHOUT CLARIN, turbid blood serum five hours after a fat meal: This unretouched dark-field photomicrograph (2500X) shows potentially hazardous fat concentrations circulating in the blood stream of a patient after a standard fat meal.

CLARIN is sublingual heparin potassium. One mint-flavored tablet taken after each meal effectively "causes a marked clarification of postprandial lipemic serum."¹ Clarin facilitates the normal physiologic breakdown of fats, with no effects on the blood-clotting mechanism.² It therefore provides important benefits for your postcoronary patients.

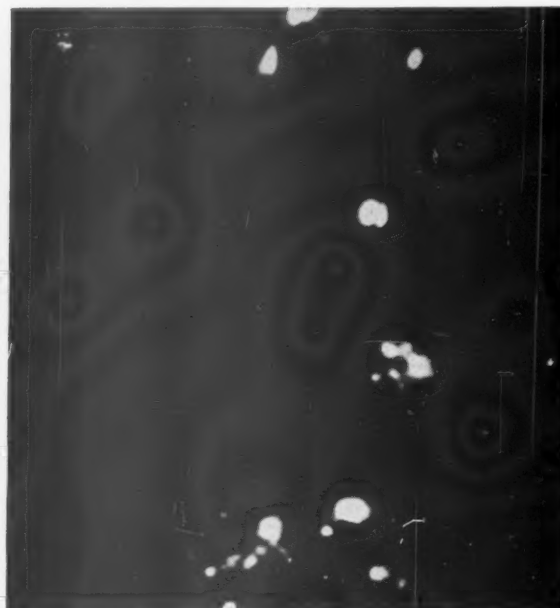
Indication: For the management of hyperlipemia associated with atherosclerosis.

Dosage: After each meal, hold one tablet under the tongue until dissolved.

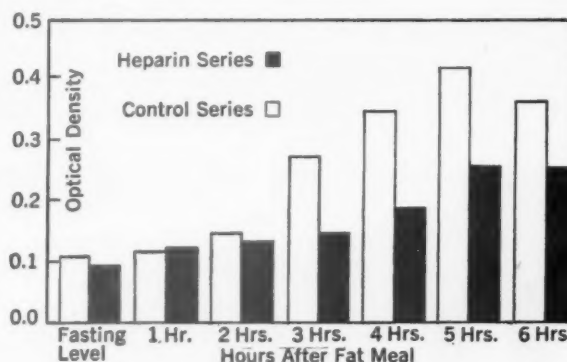
Supplied: In bottles of 50 pink, sublingual tablets, each containing 1500 I.U. heparin potassium.

1. Fuller, H. L.: *Angiology* 9:311 (Oct.) 1958.

2. Shaftel, H. E., and Selman, D.: *Angiology* 10:131 (June) 1959.



WITH CLARIN, clear blood serum five hours after a fat meal: After eating a standard fat meal as at left, the same patient has taken one sublingual Clarin tablet. Note marked clearing effect and reduction in massive fat concentrations in this unretouched photomicrograph (2500X).



Average serum optical density in 36 patients after fat meal with and without sublingual heparin.²

*Registered trade mark. Patent applied for.

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NEW ILOSONE[®] 125 SUSPENSION

Lauryl Sulfate

deliciously flavored • decisively effective

Formula:

Each 5-cc. teaspoonful provides Ilosone Lauryl Sulfate equivalent to 125 mg. erythromycin base activity.

Usual Dosage:

| | | |
|-----------------|--------------------------------|-------------------------|
| 10 to 25 pounds | 5 mg. per pound of body weight | } every six hours |
| 25 to 50 pounds | 1 teaspoonful | |
| Over 50 pounds | 2 teaspoonfuls | |

In more severe infections, these dosages may be doubled.

Supplied:

In bottles of 60 cc.

Ilosone[®] (propionyl erythromycin ester, Lilly)

Ilosone[®] Lauryl Sulfate (propionyl erythromycin ester lauryl sulfate, Lilly)

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The American Journal of Medicine

VOL. XXVII

DECEMBER, 1959

No. 6

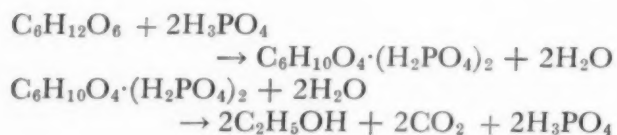
Introductory Remarks to Symposium

“ENZYMES are organic catalysts produced in, or through the agency of living cells of both animals and plants. As catalysts, enzymes are substances which vary the rate of chemical reactions, usually in the direction of speeding them up, without at the same time being themselves finally altered by the reaction in question. The majority of chemical transformations in the animal body are reactions of this nature; for example the sundry processes in the digestion of foods into simpler products, the formation from these of tissue elements, the various phenomena accompanying the activity of muscle and nerve, the production of animal heat and the numerous chemical changes involved in growth and reproduction. Even bodily dissolution after death is due to enzyme activity. A normal living organism is an orderly integrated succession of enzyme reactions.” (Chambers Encyclopaedia [1].)

Historical. The apparently spontaneous changes observed in nature, such as decomposition of vegetable and animal matter, the putrefaction of urine, the souring of milk, and the formation of intoxicating liquids from sweet ones must have been the first examples of enzymic action observed by early man. But these changes in the physical form of matter and in its properties do not appear to have aroused much speculation among thinkers in classical times. The processes of fermentation in the preparation of wine, vinegar, beer and bread, and in the souring of milk to make curd and cheese seem to have been taken for granted. The speculations of Greek and Roman writers on the nature of matter and on the changes observed in it do not seem to have advanced much further than the theorising of Lucretius [2] in regard to “atoms” and the “hooks” which hold them together.

During mediaeval times, and up until about the nineteenth century, all these reactions were thought to occur either spontaneously or in the presence and with the cooperation of living matter. With Wöhler’s synthesis of urea in 1828 a remarkable change in current thought was brought about. It is difficult for us, in the middle of the twentieth century, to appreciate what a profound effect this had on philosophy as well as science [3]. Up to this time no so-called “organic” substance had ever been observed to be formed by any agency other than a biological one; and now Wöhler had produced a typical animal metabolite by simple chemical processes carried out in the test tube. Scientific opinion now swung violently away from the idea that a vital force was necessary for those changes in matter which are associated with living organisms. In 1680 Leeuwenhoek had developed a microscope sufficiently good to reveal yeast cells and bacteria. Unfortunately he did not recognize that they were living objects. But in 1803 Thénard [4] maintained that yeast was the cause of fermentation. Later workers, e.g., de la Tour [13], Schwann [5] and Kützing [6] and Meyer [13] appeared to have settled the matter; but von Liebig [7] would have none of it. He attacked the theory that fermentation was caused by living organisms, and insisted on a theory of mechanical decomposition. Decomposition and fermentation resulted from a transfer of molecular motion from one substance to another substance or substances whose parts are held only loosely together. Thereupon ensued the well known controversy between von Liebig and Pasteur, from which Pasteur [8] emerged as the universally acclaimed victor. There was now no doubt in any one’s mind that the production of alcohol from sugar required the presence of a living agency. But the nucleus of truth in von

Liebig's contention was soon revealed. In 1858 Traube [9] maintained that the active cause of fermentation was an organic catalyst, or catalysts, contained in the yeast, but not due to the yeast cells themselves. It was about this time that the term "ferment" was replaced by that of "enzyme," which was used in almost exactly the same connotation as we use it today. In 1897 Buchner [10] demonstrated that the enzyme activity of yeast could be extracted from the yeast cells and employed extracellularly to ferment sugar to alcohol. Harden and Young confirmed and extended this finding [11]. Their well known equations, although no longer adequate to describe all that goes on during fermentation in the light of present knowledge, are nevertheless a true summary of what takes place. Particularly as modified by Meyerhof [12] they illustrate the essential enzymic nature of the reactions, in that phosphoric acid is added to sugar, with the elimination of water, and the resulting sugar phosphate then breaks up into free phosphate and something different than the sugar with which it was combined, i.e., alcohol and carbon dioxide.



Parallel to the advance in knowledge of fermentation came a series of discoveries and ideas which finally demonstrated the essentially enzymatic nature of digestion. Man cannot absorb his food unless it is reduced to small dimensions. This reduction was thought to be brought about by mechanical means until about the seventeenth century. But in that century van Helmont suggested that digestion was a chemical rather than a mechanical process. In 1752 Réaumur [13] described how different kinds of food in small perforated metal tubes introduced into the gullet of a buzzard dissolved out of the tube when it was removed from the stomach or when the bird regurgitated it. This, he said, was due to a solvent action of the stomach juices. Spallanzani [13] confirmed the fact that food became liquefied in the stomach of birds and animals. A little later the Canadian physician Beaumont [1] identified, in the gastric juice of a patient with a gastric fistula caused by a gunshot wound, the digestive enzyme pepsin, which in 1836 Schwann [14] obtained in a com-

paratively uncontaminated state. Kirchoff [15] converted starch to sugar with an extract of wheat in 1814; Payen and Persoz [16] observed a similar action by malt extract, and were able to precipitate the active catalytic material from it by alcohol precipitation. To it they gave the name which we still use today, i.e., diastase. In the eighteen fifties Bernard [17] and his colleagues did their classic work on saliva and pancreatic juice and the actions of their enzymes on carbohydrates and proteins. The starch-splitting enzyme (diastase, later called amylase) was separated from the protein-splitting enzyme of pancreatic juice by Danilewsky in 1862 [18].

Enzymes in Clinical Biochemistry. All this early work on enzymes led inevitably to the finding that enzymes are concerned in pathologic processes, as well as in normal physiologic ones, and that their study in pathologic conditions may be a valuable addition to our armoury of investigations of disease. Clinical abnormalities, brought about by invasive, destructive or deficiency causes, exhibit the patterns of signs and symptoms familiar to the physician; but they also exhibit patterns of biochemical abnormality, in which abnormalities of concentration of one or more substances in various fluids or sites of the body may be as distinguishing a feature of the disease as the longer known and better recognized criteria on which the clinician has traditionally based his diagnosis. The absence of hydrochloric acid and pepsin from the gastric juice of persons with pernicious anaemia is as invariable a sign of the disease as their pallor. The elevated blood urea of a patient with uraemia is as characteristic as his odour. Enzyme abnormalities are as common and as useful as those of other constituents of the blood and other body fluids.

It was with the digestive enzymes that abnormalities of a possibly useful clinical sort were first looked for. Pavlov's researches with gastric secretion had demonstrated differences in the peptic activity of gastric juice, and the simple estimation of this activity with the Mett tube (a glass tube filled with coagulated albumin, closed at one end and open at the other, into which pepsin eats its way at a rate proportional to its activity or concentration) furnished a ready means of estimating the peptic activity of aspirated gastric juice from clinical cases [19]. Although no longer used, this was a commendable early effort to apply biochemistry to diagnostic purposes. A more successful one was

that of estimating diastase in the urine of patients with acute pancreatitis. The well known diastase units of Wohlgemuth expressed the enzyme activity of urine in terms related to the dilution with water at which the urine would fully digest a solution of starch. Very high values of diastase were found in the urine of patients with acute pancreatitis, as compared with those of normal persons or patients suffering from other diseases.

From the studies on fermentation came the first clue that the phosphate combining and splitting enzymes might be involved with processes in the animal body as well as in the yeast cells. Robison [20] demonstrated a high concentration of a glucose-phosphate splitting enzyme, which he called phosphatase, in young growing bones, and a still higher concentration in rachitic bones. On this he based his well known phosphatase theory of bone formation. Kay [27] found that the high levels of phosphatase activity in growing bones were reflected by a higher activity of the enzyme in the blood plasma of infants and children than in that of adult persons, and that the phosphatase activity of the plasma of rachitic children was still higher, as likewise that of the plasma of patients with adult rickets, osteomalacia and other generalized bone diseases, e.g., Paget's disease and osteitis fibrosa cystica.

Since those early days the complexity of enzyme investigation for diagnostic, prognostic and other clinical purposes has enormously increased. The confirmation of diagnosis of a disease, or even the establishment of it, by enzyme investigation has become commonplace. Determinations of the levels of enzyme activity frequently yield valuable information as to the severity of the disease and the prognosis for the patient; their estimation is almost essential for assessment of the efficacy of some forms of therapy.

General Properties of Enzymes on Which Their Determination is Based. Some general properties of enzymes and principles governing their assessment should be emphasized. The measurement of enzyme activity is in some respects a different problem from that of the determination of a defined chemical substance. I stress the word "activity" rather than amount or concentration of an enzyme, because it is really the work which an enzyme will perform in catalyzing a chemical reaction that we measure, not the actual amount of the enzyme which is present. Indeed, for most

enzymes we have no possible means of determining their amount; we can only measure their catalytic activity, i.e., in terms of the increase in the rate of a chemical reaction which they will bring about. While many enzymes have been purified and even crystallized, this has been done with very few of clinical interest. With aldolase (the enzyme which catalyses the cleavage of fructose-1:6-diphosphate into glyceraldehyde phosphate and dihydroxy-acetone phosphate) it is possible to compare the enzyme activity of a measured amount of a biological fluid with that of a weighed amount of the crystalline enzyme, and on the basis of their comparative activities to arrive at a conclusion as to the probable actual concentration of the enzyme in the biological fluid. But with the great majority of enzymes useful to the clinician there is no such basis for a comparison. The activity of the enzyme in the blood plasma or other body fluid or tissue must therefore be expressed in some arbitrary terms, such as the amount of some easily determinable substance whose hydrolysis, oxidation or reduction the enzyme will catalyse. More often, the amount of a product of the reaction catalysed by the enzyme is determined; thus, for instance, an amount of blood plasma which will catalyse the hydrolysis of a phosphoric ester to give 1 mg. of free phosphate, under a set of standard conditions, can be said, arbitrarily, to contain one "unit" of phosphatase activity. Similarly, for other enzymes the amount of a product which has been formed in a catalysed chemical reaction, or the amount of a substrate which has disappeared during that catalysed reaction, is taken as a measure of the enzyme's activity in the material under investigation.

Many factors other than the concentration of an enzyme govern its activity. The substrate concentration is critical: generally speaking the greater the amount of substrate the more quickly will the reaction be driven towards the product; but in order to get reproducible and comparable results the concentration of substrate must always be kept the same in any set of determinations of enzyme activity. Temperature plays an important part in enzyme-catalysed reactions: up to some temperature at which there is heat destruction of the enzyme there is an increase in the rate of the catalysed reaction. Temperature must therefore be carefully controlled; 37°C. is the most convenient, though not always the best, temperature at which to conduct enzyme studies.

TABLE I
ENZYMES IN DIAGNOSIS

| Enzyme (where determined) | Units (definition) | Normal Range (units) | Clinical Conditions in Which Abnormal Values Are Found |
|-----------------------------|--|--|--|
| <i>Digestive Enzymes</i> | | | |
| Amylase: | | | |
| Urine | = ml. 0.1% starch digested by 1 ml. in 30 min. [25] | 6-30 [26] | Acute pancreatitis, >200 [26] Neoplasm of pancreas, 30-100 Chronic pancreatitis, 10-50 |
| Plasma | = enzyme which destroys 5 mg. starch in 15 min. [26,27] | 70-200 [26] | Acute pancreatitis, 200-1,000 [28] Chronic pancreatitis, 200-400 Mumps, 126-740 [29,30] |
| Lipase: | | | |
| Plasma | = ml. 0.05 N NaOH needed to neutralize free fatty acid hydrolysed in 4 hours from olive oil [37] | 0.06-0.87 per ml. | Acute pancreatitis, 1.5-3 Chronic pancreatitis, slight rise Carcinoma of pancreas, slight rise Cholecystitis, slight rise [32,33] |
| Trypsin: | | | |
| Faeces (of infants) | Liquefaction of gelatin [26,34] | Dilution of 1 in 100 | Fibrocystic disease, dilution of 1 in 50 or less [35] |
| Duodenal juice (children) | Liquefaction of gelatin | Dilution of >1 in 50 | Fibrocystic disease, dilution of <1 in 12 [36,37] |
| Pepsin: | | | |
| Gastric juice | = mg. tyrosine liberated from haemoglobin as substrate in 10 min. [38,39] | Males, 522×10^3 per 24 hours Females, 342×10^3 [40] | Raised in duodenal ulcer [40] Low or absent in pernicious anemia [41-43] |
| Plasma | = μ g. tyrosine liberated from haemoglobin in 24 hours [44] | Males, 461 ± 19 per ml. plasma Females, 354 ± 22 | Raised values in duodenal ulcer |
| Peptidase: | | | |
| Liver | See original articles [45] | | Raised in hepatitis [46] |
| Uropepsin: | | | |
| Urine | = mg. tyrosine liberated from haemoglobin substrate in 10 min. [44] | Males, 73 ± 8 per 24 hours Females, 41 ± 6 [40] | Raised in duodenal ulcer, may correlate with gastric pepsin and acidity [47,48] |
| <i>Phosphomonoesterases</i> | | | |
| Alkaline phosphatase: | | | |
| Plasma | Bodansky: mg. inorg. P hydrolysed from glycerophosphate in 1 hour [49-51] King-Armstrong, mg. phenol hydrolysed from phenylphosphate in 15 min. [52,26] | 1.5-5 [51] 3-13 in adults [52] 11-20 in children [53] | Rickets, 8-16 Bodansky units, 20-40 K-A units [54,55] Osteomalacia, 8-16 Bodansky units, 20-40 K-A units [54,55] Hyperparathyroidism, 8-100 Bodansky units, 20-200 K-A units Osteitis deformans, 8-100 Bodansky units, 20-200 K-A units Metastatic carcinoma, 5-50 Bodansky units, 15-250 K-A units Osteogenic sarcoma, 5-50 Bodansky units, 15-250 K-A units Obstructive jaundice, >12 Bodansky units, >30 K-A units, partial 15-30 [56-58] Hepatitis, 6-12 Bodansky units, 15-30 K-A units [26,59] Cirrhosis, 6-12 Bodansky units, 15-30 K-A units Hypophosphatasia, very low or absent [60-65] |

TABLE 1 (Continued)
ENZYMES IN DIAGNOSIS

| Enzyme (where determined) | Units (definition) | Normal Range (units) | Clinical Conditions in Which Abnormal Values Are Found |
|---|---|----------------------|---|
| Glucose-6-phosphatase: Liver Plasma | Inorg. P hydrolysed from glucose-6-phosphate [66] = $\mu\text{g. P}$ hydrolysed in 1 hour [68] | <20 per ml. | Very low in glycogen storage disease [66,67] Very low in glycogen storage disease Hepatitis and cirrhosis, raised 2-20 times normal [68] |
| Acid phosphatase: Plasma, total | (1) mg. inorg. P hydrolysed from glycerophosphate [69,70] (2) mg. phenol hydrolysed from phenylphosphate in 1 hour at pH 5 [26,71] | 0-4 | Carcinoma of prostate, 5-2000 units total [71-73], 1-2,000 units tartrate-labile [76] |
| Plasma, tartrate-labile | As in (2) but difference between hydrolysis with and without d-tartrate [74-76] | 0-0.7 | Non-malignant prostatitis, normal except after palpation (4-8 total) [41,77,78] Paget's disease, total slightly raised [79], tartrate-labile normal [76] Breast carcinoma, total slightly raised, tartrate-labile normal [76] Gaucher's disease, total slightly raised, tartrate-labile normal [76,80] |

Glycolytic Enzymes

| | | | |
|------------------------------------|--|---|--|
| Aldolase: Plasma | (1) microlitres of fructose-diphosphate split into glyceraldehyde phosphate and dihydroxyacetone phosphate in 1 hour [87] (2) mg. P as triose phosphate per min. [82] | 2-9.6 per ml. [87] Adults, 0.2 ± 0.03 Children, 0.4 ± 0.03 [82] | Progressive muscular dystrophy, 2.7-4 (2) units in children, 0.9-2 in adults [82-84] Myocardial infarction, transient increases of 6-31 units [85] Acute liver necrosis, high values [86] Obstructive jaundice, normal [86] Cancer, general, some raised [87] Carcinoma of prostate, 12 to 24 [97] units, decreased with estrogen therapy [88,89] |
| Phosphohexose-isomerase: Plasma | = 25 $\mu\text{g. fructose}$ (as fructose-6-phosphate) from glucose-6-phosphate in 30 min. per ml. [90] | 8-40 [90] | Progressive muscular dystrophy, raised Myocardial infarction, transient increases [85] Haemolytic anaemia, raised Carcinoma of prostate, raised, particularly with metastases; roughly parallels acid phosphatase [97] Carcinoma of breast, high values, particularly with metastases [92] |
| Phosphoglucomutase: Plasma | $10^2 \times \mu\text{moles glucose-6-phosphate}$ formed from glucose-1-phosphate in 4 hours [93] | 46 ± 17 [93] | Cancer, raised [93] Hepatitis, raised [94] |

TABLE I (Continued)
ENZYMES IN DIAGNOSIS

| Enzyme (where determined) | Units (definition) | Normal Range (units) | Clinical Conditions in Which Abnormal Values Are Found | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|--|--|--|--|------|------|----------------------|-------|--------|------------------------|--------|--------|--------------------------------|-----------|-----------|---------------------|--------|--------|-------------------------|--------|---------|---------------------------|--------|-------|------------------------------------|--------|--|--------------------|-----------|--|
| Transaminases | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glutamic-oxaloacetic transaminase (GO-T): Serum | (1) photometric, = 0.001 O.D. * per min. with DPNH, at 340 mμ. [95-98] | GO-T 20 ± 7 GP-T 16 ± 9 [103] | Myocardial infarction, GO-T greatly increased, up to and greater than 200 units, GP-T moderately increased [96,105-107] | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glutamic-pyruvic transaminase (GP-T): Serum | (2) colorimetric with 2:4-dinitrophenylhydrazine = μg. pyruvate formed [99-102] | GO-T 8-40 GP-T 5-30 [103,104] | Liver disease, GO-T and GP-T raised to various extents [108-117] | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | <table><tr><td></td><td>GO-T</td><td>GP-T</td></tr><tr><td>Obstructive jaundice</td><td>44-88</td><td>64-400</td></tr><tr><td>Intrahepatic carcinoma</td><td>43-300</td><td>26-240</td></tr><tr><td>Infectious hepatitis [108-111]</td><td>460-2,140</td><td>600-2,600</td></tr><tr><td>Cirrhosis [109,112]</td><td>45-300</td><td>20-258</td></tr><tr><td>Drug jaundice [109,112]</td><td>68-370</td><td>176-440</td></tr><tr><td>Haemolytic jaundice [113]</td><td>32-140</td><td>20-40</td></tr><tr><td>Muscular dystrophies [110,114-116]</td><td>Raised</td><td></td></tr><tr><td>Acute pancreatitis</td><td>Up to 600</td><td></td></tr></table> | | GO-T | GP-T | Obstructive jaundice | 44-88 | 64-400 | Intrahepatic carcinoma | 43-300 | 26-240 | Infectious hepatitis [108-111] | 460-2,140 | 600-2,600 | Cirrhosis [109,112] | 45-300 | 20-258 | Drug jaundice [109,112] | 68-370 | 176-440 | Haemolytic jaundice [113] | 32-140 | 20-40 | Muscular dystrophies [110,114-116] | Raised | | Acute pancreatitis | Up to 600 | |
| | GO-T | GP-T | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Obstructive jaundice | 44-88 | 64-400 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Intrahepatic carcinoma | 43-300 | 26-240 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Infectious hepatitis [108-111] | 460-2,140 | 600-2,600 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cirrhosis [109,112] | 45-300 | 20-258 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Drug jaundice [109,112] | 68-370 | 176-440 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Haemolytic jaundice [113] | 32-140 | 20-40 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Muscular dystrophies [110,114-116] | Raised | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acute pancreatitis | Up to 600 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| The Dehydrogenases | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lactic dehydrogenase: Serum | DPNH ₂ change in optical density of 0.001 per min. at 340 mμ. [117,118] | 100-600 per ml. [114] | Myocardial infarction, increased 4 to 5 times, elevated longer than GO-T [85, 103, 119, 120] Hepatitis, acute viral, moderately increased [121,122] Muscular dystrophy, moderately increased [123] Carcinoma, metastatic, raised [124-127] Leukaemia, raised [128] | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malic dehydrogenase: Plasma | DPNH ₂ change in optical density of 0.001 per min. at 340 mμ. [129] | 25-100 per ml. [129] | Myocardial infarction, increased [128-130] | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Isocitric dehydrogenase: Plasma | = mμmole TPNH ₂ per hr. [131] | 55-224 per ml. [121,131] | Myocardial infarction, normal [121,132] Hepatitis, acute viral, up to 4,500 [132] Hepatitis, chronic viral, less elevated but for longer periods [132] Carcinoma, metastatic, normal | | | | | | | | | | | | | | | | | | | | | | | | | | | |

* Change in optical density of 0.001.

TABLE I (Continued)
ENZYMES IN DIAGNOSIS

| Enzyme (where determined) | Units (definition) | Normal Range (units) | Clinical Conditions in Which Abnormal Values Are Found |
|--|---|------------------------------|---|
| Glucose-6-phosphate dehydrogenase: R.B.C. | μ mole glucose-6-phosphate utilized per hr. per 10 second R.B.C. | 848 [133] | Anaemia, haemolytics decreased [133] Hepatitis, raised [133] Obstructive jaundice, raised [133] |
| <i>Miscellaneous Enzymes</i> | | | |
| Glutathione reductase: Plasma | DPNH ₂ change in optical density of 0.001 per min. at 340 m μ . [135] | 10-70 per ml. | Hepatitis, raised Hepatic coma, raised Carcinoma, generalized, raised Myocardial infarction, normal |
| Cholinesterases: Pseudo, in plasma | = drop in pH \times 100 from pH 8.1 in 1 hr. due to hydrolysis of acetic acid from acetyl choline [136-142] | 60-126 per 100 ml. [136,143] | Liver disease [143,144] Cirrhosis, decreased to about half Hepatitis, decreased to about half Obstructive jaundice, slightly decreased |
| True, erythrocytes | = drop in pH \times 100 from pH 8.1 in 1 hr. due to hydrolysis of acetic acid from acetyl choline [136-142] | | Insecticide poisoning with organophosphorus compounds, markedly lowered [145,146] |
| Caeruloplasmin: (Serum copper oxidase) | Change in optical density of <i>p</i> -phenylenediamine at 530 m μ . [147,148] | 0.1-0.3 per 0.1 ml. | Wilson's disease (hepatolenticular degeneration) < 0.05 [149,152] |

Hydrogen ion concentration is as important as temperature. Most enzymes have an optimal pH, which must be maintained by means of a buffer system, but the buffer must be carefully picked in order not to introduce any inhibiting influence on the enzyme. Coenzymes and activators are necessary for many enzymes, and these may have to be added to the mixture of enzyme, substrate and buffer in order to determine the true assessment of the real enzyme activity. On the other hand, inhibitors or antagonists of enzyme action must be watched for. A simple illustration will suffice: undiluted urine has less apparent phosphatase than highly diluted urine, but dialysed undiluted urine shows the same high activity as the diluted urine; here an inhibitor has been eliminated by dialysis, on the one hand, and diluted out of effective concentration on the other. The instability of enzymes must be watched: they are destroyed by heat, by unfavourable hydrogen ion concentration and other factors; certain substances, e.g., heavy metals,

combine irreversibly with them and destroy their activity; leaving blood plasma in a warm place and allowing it to become alkaline in reaction through loss of CO₂ will lead to the destruction of acid phosphatase; improperly cleaned glassware may contain inhibiting or destructive substances. The activity of an enzyme under investigation may easily be added to by contamination from an accompanying tissue, e.g., the acid phosphatase of the erythrocytes may greatly increase the acid phosphatase activity of plasma if appreciable haemolysis takes place, and several enzymes may be discharged from the platelets into the blood plasma if they are treated roughly, thus increasing the apparent activity of the plasma for these particular enzymes.

For comprehensive statements on the nature of enzymes and enzyme action, of their kinetics and their measurement, the reader is referred to the following article by Dr. O. Bodansky [22], and to the excellent books by Dixon and Webb on

"Enzymes" [23] and "Methods in Enzymology" by Colowick and Kaplan [24].

In Table 1 a brief review is given of most of the enzymes which have become of clinical importance in recent years. These include the digestive enzymes, the phosphomonoesterases, the glycolytic enzymes, those associated with the Krebs cycle, and several miscellaneous enzymes. No attempt will be made here to discuss them fully.

SUMMARY

The history of enzymes and of the ideas concerning them in relation to physiologic processes is reviewed. The importance of enzymes in pathologic processes, and the great usefulness of their determination in body fluids in relation to diagnosis, prognosis and in the assessment of therapy is pointed out.

The enzymes most commonly used in diagnosis are listed, the methods for their determination briefly indicated, and the range of activities in normal blood plasma and other fluids stated. The clinical conditions in which abnormalities have been found are given for each enzyme, together with some indication as to the size of the change and its direction.

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REFERENCES

1. Chambers Encyclopaedia; New ed., p. 362. London, 1955. George Newnes.
2. LUCRETIVS. De rerum natura. In: The Nature of the Universe. London, 1952. Penguin Classics.
3. HOPKINS, F. G. The centenary of Wöhler's synthesis of urea, 1828-1928. *Biochem. J.*, 22: 1314, 1928.
4. THÉNARD, L. J. Mémoire sur la fermentation vineuse. *Ann. Chim. phys.*, 46: 294, 1803.
5. SCHWANN, T. Vorläufige Mitteilung, betreffend Versuche über die Weingärung und Fäulnis. *Ann. Physik.*, 41: 184, 1837.
6. KÜTZING, F. Mikroskopische Untersuchungen über die Hefe und Essigmutter, nebst mehreren anderen dazugehörigen vegetabilischen Gebilden. *J. prakt. Chem.*, 11: 385, 1837.
7. VON LIEBIG, J. Ueber die Erscheinungen der Gärung, Fäulnis und Verwesung und ihre Ursachen. *Liebig's Ann.*, 30: 250, 1839.
8. (a) PASTEUR, L. Mémoire sur la fermentation alcoolique. *Ann. Chim. Phys.*, 58: 323, 1860.
(b) IDEM. Note sur la mémoire de M. Liebig relatif aux fermentations. *Ann. Chim. Phys.*, 25: 145, 1872.
9. TRAUBE, M. Theorie der Fermentwirkungen. Berlin, 1858. Ferd. Dummler's Verlagsbuchh.
10. BUCHNER, E. Alkoholische Gärung ohne Hefezellen. *Ber. d. deutsch. chem. Gesellsch.*, 30: 117, 1110, 1897. *Ber.*, 30: 117, 1110, 1897.
11. (a) HARDEN, H. and YOUNG, W. J. Gärversuche mit Presssaft aus obergäriger Hefe. *Ber. d. deutsch. chem. Gesellsch.*, 37: 1052, 1904.
(b) IDEM. The influence of phosphates on the fermentation of glucose by yeast-juice. *Proc. Chem. Soc.*, 21: 189, 1905.
12. MEYERHOF, O. Die chemischen Vorgänge im Muskel und ihr Zusammenhang mit Arbeitsleistung und Wärmebildung. Berlin, 1930. Julius Springer.
13. Encyclopaedia Britannica. London.
14. SCHWANN, T. Ueber das Wesen der Verdauungsprocessus. *Arch. f. Anat., Physiol. u. wissensch. Med.*, p. 90, 1836.
15. KIRCHOFF, C. Ueber die Reinigung der Getreidestärke. *Schweigger's J. f. Chem. u. Physik.*, 14: 385, 1815.
16. PAYEN, A. and PERSOZ, Mémoire sur la diastase, les principaux produits de ses reactions et leur applications aux arts industriels. *Ann. Chim. phys.*, 53: 73, 1833.
17. BERNARD, C. Mémoire sur le Pancreas. Paris, 1856. Ballière.
18. DANILEWSKY, A. Über specifisch wirkende Körper des natürlichen und künstlichen pankreatischen Safts. *Arch. Path. Anat. & Physiol.*, 25: 279, 1862.
19. BABKIN, B. P. Secretory Mechanism of the Digestive Glands, 2nd ed. London, 1950. Cassell & Co.
20. ROBISON, R. The Significance of Phosphoric Esters in Metabolism. New York, 1932. New York University Press.
21. (a) KAY, H. D. The phosphatase of mammalian tissues. *Biochem. J.*, 22: 855, 1928.
(b) Plasma phosphatase in osteitis deformans and in other diseases of bone. *Brit. J. Exper. Path.*, 10: 253, 1929.
(c) Plasma phosphatase. 1. Method of determination. *J. Biol. Chem.*, 89: 235, 1930.

General Properties of Enzymes

22. BODANSKY, O. Diagnostic applications of enzymes in medicine: general enzymological aspects. *Am. J. Med.*, 27: 861, 1959.
23. DIXON, M. and WEBB, B. Enzymes. London, 1958. Longmans, Green & Co.
24. COLOWICK, S. P. and KAPLAN, N. O. Methods in Enzymology. New York, 1955. Academic Press.

Enzymes in Diagnosis

Amylase

25. WOHLGEMUTH, J. Über eine neue Methode zur quantitativen Bestimmung des diastaphischen Fermens. *Bioch. Ztschr.*, 9: 1, 1908.

26. KING, E. J. and WOOTTON, I. D. P. Micro-analysis in Medical Biochemistry, 3rd ed. London, 1959. Churchill.
27. SOMOGYI, M. Diastatic activity of human blood. *Arch. Int. Med.*, 67: 665, 1941.
28. McCORKLE, H. and GOLDMAN, L. Clinical significance of serum amylase test in diagnosis of acute pancreatitis. *Surg., Gynec. & Obst.*, 74: 439, 1942.
29. CANDEL, S. and WHEELLOCK, M. C. Serum amylase and serum lipase in mumps. *Ann. Int. Med.*, 25: 88, 1946.
30. WARREN, W. R. Serum amylase and lipase in mumps. *Am. J. M. Sc.*, 30: 161, 1955.

Lipase

31. BUNCH, L. D. and EMERSON, R. L. Serum lipase determination; 4 hour technic with olive oil substrate. *Clin. Chem.*, 2: 75, 1956.
32. CHERRY, I. S. and CRANDALL, L. A. Specificity of pancreatic lipase: its appearance in blood after pancreatic injury. *Am. J. Physiol.*, 100: 266, 1932.
33. HENRY, R. S., SOBEL, C. and BERKMAN, S. On the determination of pancreatitis lipase in serum. *Clin. Chem.*, 3: 77, 1957.

Trypsin

34. GORDON, I. G., LEVIN, B. and WHITEHEAD, T. P. Estimation of trypsin in duodenal juice. *Brit. M. J.*, 1: 463, 1952.
35. PAYNE, W. W. Laboratory tests in diagnosis of coeliac and fibrocystic disease. *Great Ormond St. J.*, 3: 23, 1952.
36. BODIAN, M. Fibrocystic Disease of the Pancreas. London, 1952. Heineman.
37. AGREN, G., LANGERLÖF, H. and BERGLUND, H. Secretin test of pancreatic function in diagnosis of pancreatic disease. *Acta med. Scandinav.*, 90: 224, 1936.

Pepsin

38. ANSON, M. L. and MIRSKY, A. E. Estimation of pepsin with haemoglobin. *J. Gen. Physiol.*, 16: 59, 1932.
39. BUCHER, G. R., GROSSMAN, M. I. and IVY, A. C. Pepsin method; role of dilution in determination of peptic activity. *Gastroenterology*, 5: 501, 1945.
40. VAN GOIDSENHOVEN, G. V., WILKOFF, L. and KIRSNER, J. B. Serum and urine pepsinogen and gastric pepsin: simultaneous analyses for 24 hour periods in normal persons and in patients with duodenal ulcer, gastric ulcer and achlorhydria. *Gastroenterology*, 34: 421, 1958.
41. KING, E. J. Enzymes in clinical biochemistry. *Clin. Chem.*, 3: 507, 1957.
42. MOLLIN, D. L., BAKER, S. J. and DONIACH, I. Addisonian pernicious anaemia in gastric atrophy in young man. *Brit. J. Haemat.*, 1: 278, 1955.
43. POLINER, I. J. and SPIRO, H. M. The independent secretion of acid pepsin and intrinsic factor by the human stomach. *Gastroenterology*, 34: 195, 1958.
44. MIRSKY, I. A., BLOCK, S., OSHER, S. and BROHKAHN, R. H. Uropepsin excretion by man: source, properties and assay of uropepsin. *J. Clin. Invest.*, 27: 818, 1948.

Peptidases

45. (a) FLEISHER, G. A. Peptidases in human serum. A comparison of activities between normal persons and patients with liver diseases and other pathologic conditions. *J. Clin. Invest.*, 32: 674, 1953.
- (b) Peptidases in human blood. II. The hydrolysis of additional peptides by normal serum. *J. Biol. Chem.*, 206: 637, 1954.
- (c) Peptidases in human leukocytes. *Ann. New York Acad. Sc.*, 59: 1012, 1955.
- (d) Peptidases in human blood. IV. The hydrolysis of glycl-L-leucine and other dipeptides by leukocytes. *Arch. Biochem. Biophys.*, 61: 119, 1956.
46. FLEISHER, G. A., BUTT, H. R. and HUIZINGER, K. A. Enzymatic hydrolysis of L-leucyl-glycine in serum in hepatic disease. *Proc. Staff Meet., Mayo. Clin.*, 32: 410, 1957.

Uropepsin

47. SIRCUS, W. Studies of uropepsinogen excretion in gastrointestinal disorders. *Quart. J. Med.*, 23: 291, 1954.
48. NYHUS, L. M. Uropepsin excretion: its relation to duodenal ulcer disease in diagnosis and therapy. *Surgery*, 41: 406, 1957.

Alkaline phosphatase

49. KAY, H. D. Plasmaphosphatase; method of determination; some properties of the enzyme. *J. Biol. Chem.*, 89: 235, 1930.
50. JENNER, H. D. and KAY, H. D. Plasma phosphatase; clinical method for determination of plasma phosphatase. *Brit. J. Exper. Path.*, 13: 22, 1932.
51. BODANSKY, O. and BAKWIN, H. Factors influencing measurement of phosphatase activity of tissue extracts. *J. Biol. Chem.*, 101: 641, 1933.
52. KING, E. J. and ARMSTRONG, A. R. A convenient method for determining serum and bile phosphatase activity. *Canad. M. A. J.*, 31: 376, 1934.
53. GRAY, J. D. and CARTER, F. S. Early diagnosis of rickets. *Arch. Dis. Child.*, 24: 189, 1949.
54. KAY, H. D. Phosphatase in growth and in disease of bone. *Physiol. Rev.*, 12: 384, 1932.
55. THOMPSON, R. H. S. and KING, E. J. Biochemical Disorders in Human Disease. New York, 1957. Academic Press.
56. ROBERTS, W. M. Blood phosphatase and van den Bergh reaction in differentiation of several types of jaundice. *Brit. M. J.*, 1: 734, 1933.
57. BODANSKY, O. and JAFFE, H. L. Phosphatase studies; serum phosphatase in diseases of bone: interpretation and significance. *Arch. Int. Med.*, 54: 88, 1934.
58. ARMSTRONG, A. R., KING, E. J. and HARRIS, R. L. Phosphatase in obstructive jaundice. *Canad. M. A. J.*, 31: 14, 1934.
59. HERBERT, F. K. Plasma phosphatase in various types of jaundice. *Brit. J. Exper. Path.*, 16: 365, 1935.
60. SCAGLIONE, P. R. and LUCEY, J. F. Further observations on hypophosphatasia. *Dis. Child.*, 92: 403, 1956.
61. CURRARINA, G., NEUHAUSER, E., REYERSBACH, G. C.

- and SOBEL, E. Hypophosphatasia. *Am. J. Roentgenol.*, 78: 392, 1957.
62. FRASER, D., YENDT, E. R. and CHRISTIE, F. H. E. Metabolic abnormalities in hypophosphatasia. *Lancet*, 1: 286, 1955.
 63. McCANCE, R. A., MORRISON, A. B. and DENT, C. E. Excretion of phosphoethanolamine and hypophosphatasia; preliminary communication. *Lancet*, 1: 131, 1955.
 64. CUSWORTH, D. C. The isolation and identification of phosphoethanolamine from the urine of a case of hypophosphatasia. *Biochem. J.*, 68: 262, 1958.
 65. SHERLOCK, S. Diseases of the Liver and Biliary System. Oxford, 1958. Blackwell Scientific Publications.
 - phosphatase following palpation of the prostate. *Lancet*, 1: 998, 1952.
 79. GUTMAN, A. B., TYSON, T. L. and GUTMAN, E. B. Serum calcium, inorganic phosphorus and phosphatase activity in hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic disease of the bones. *Arch. Int. Med.*, 57: 379, 1936.
 80. (a) TUCHMAN, L. R., SUNA, H. and CARR, J. Elevation of serum acid phosphatase in Gaucher's disease. *J. Mt. Sinai Hosp.*, 23: 227, 1956.
 - (b) TUCHMAN, L. R. and SWICK, M. High acid phosphatase level indicating Gaucher's disease in patient with prostatism. *J. A. M. A.*, 164: 2034, 1957.

Glucose-6-phosphatase

66. (a) SWANSON, M. A. Phosphatases of liver: glucose-6-phosphatase. *J. Biol. Chem.*, 184: 647, 1950.
- (b) SWANSON, M. A. and CORI, C. F. Studies on the structure of polysaccharides. III. Relation of structure to activation of phosphorylases. *J. Biol. Chem.*, 172: 815, 1948.
67. HARRIS, R. C. and OLMO, C. Liver and kidney glucose-6-phosphatase activity in children with normal and diseased organs. *J. Clin. Invest.*, 33: 1204, 1954.
68. KOIDE, H. and ODA, T. Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. *Clin. chim. Acta*, 4: 554, 1959.

Acid phosphatase

69. SHINOWARA, G. Y., JONES, L. M. and REINHART, H. L. The estimation of serum inorganic phosphate and acid and alkaline phosphatase activity. *J. Biol. Chem.*, 142: 921, 1942.
70. WOODARD, H. Q. Factors leading to elevations in serum acid glycerophosphatase. *Cancer*, 5: 236, 1952.
71. GUTMAN, E. B. and GUTMAN, A. B. Estimation of acid phosphatase activity of blood serum. *J. Biol. Chem.*, 136: 201, 1940.
72. HUGGINS, C. G., STEVENS, R. E. and HODGES, C. V. Studies on prostatic cancer. II. The effects of castration on advanced carcinoma of the prostate gland. *Arch. Surg.*, 43: 209, 1941.
73. WATKINSON, J. M., DELORY, G. E., KING, E. J. and HADDOW, A. Plasma acid phosphatase in carcinoma of the prostate and the effect of treatment with stilboestrol. *Brit. M. J.*, 2: 492, 1944.
74. ABUL-FADL, M. A. M. and KING, E. J. Properties of the acid phosphatases of erythrocytes and of the human prostate gland. *Biochem. J.*, 45: 51, 1949.
75. FISHMAN, W. H. and LERNER, F. A method for estimating serum acid phosphatase of prostatic origin. *J. Biol. Chem.*, 200: 89, 1953.
76. KING, E. J. and JEGATHEESAN, K. A. A method for the determination of tartrate-labile, prostatic acid phosphatase in serum. *J. Clin. Path.*, 12: 85, 1959.
77. HOCK, E. and TESSIER, R. N. Elevation of serum acid phosphatase after prostatic massage. *J. Urol.*, 62: 488, 1949.
78. DANIEL, O. and VAN ZYL, J. J. Rise of serum acid

81. SIBLEY, J. A. and LEHNINGER, A. L. Aldolase in serum and tissues of tumour-bearing animals. *J. Nat. Cancer Inst.*, 9: 303, 1949.
82. SCHAPIRA, G., DREYFUS, J. C., SCHAPIRA, F. and KRUH, J. Glycogenolytic enzymes in human progressive muscular dystrophy. *Am. J. Phys. Med.*, 34: 313, 1955.
83. ARONSON, S. M. and VOLK, B. W. Studies on serum aldolase activity in neuromuscular disorders. I. Clinical applications. *Am. J. Med.*, 22: 414, 1957.
84. EVANS, J. S. and BAKER, R. W. R. Serum aldolase and the diagnosis of myopathy. *Brain*, 80: 557, 1957.
85. WHITE, L. P. Serum enzymes. I. Serum lactic dehydrogenase in myocardial infarction. *New England J. Med.*, 255: 984, 1956.
86. SCHWARZMANN, V. Modification des transaminases et de l'aldolase sérique au cours des maladies du foie. *Arch. mal. app. digest.*, 46: 11, 1957.
87. SIBLEY, J. A. and FLEISHER, G. A. The clinical significance of serum aldolase. *Proc. Staff Meet., Mayo Clin.*, 29: 591, 1954.
88. BAKER, R. and GOVAN, D. The effect of hormonal therapy of prostatic cancer on serum aldolase. *Cancer Res.*, 13: 14, 1953.
89. WHITE, L. P. Enzymes in blood. *Ann. New York Acad. Sc.*, 75: 349, 1958.

Phosphohexose isomerase

90. BODANSKY, O. Serum phosphohexose isomerase in cancer. I. Method of determination and establishment of range of normal values. *Cancer*, 7: 1191, 1954.
91. BODANSKY, O. Serum phosphohexose isomerase in cancer; as index of tumor growth in metastatic carcinoma of prostate. *Cancer*, 8: 1087, 1955.
92. (a) BODANSKY, O. Serum phosphohexose isomerase in cancer; as index of tumor growth in metastatic carcinoma of breast. *Cancer*, 7: 1200, 1954.
- (b) MYERS, W. P. and BODANSKY, O. Comparison of serum phosphohexose isomerase activity and urinary calcium excretion in a patient with metastatic mammary carcinoma. *Am. J. Med.*, 23: 804, 1957.

Phosphoglucomutase

93. BODANSKY, O. Phosphoglucomutase activity in human serum. *Cancer*, 10: 859, 1957.

94. DE RITIS, F., GUISTI, G. and COLTORTI, M. Serum phosphoglucomutase activity in human virus hepatitis. *Experientia*, 13: 81, 1957.

Transaminases

95. KARMEN, A. A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. *J. Clin. Invest.*, 34: 131, 1955.
96. WROBLEWSKI, F. and LADUE, J. S. Serum glutamic pyruvic transaminase (SGP-T) in hepatic disease: a preliminary report. *Ann. Int. Med.*, 45: 801, 1956.
97. STEINBERG, D., BALDWIN, D. and OSTROW, B. H. A clinical method for the assay of serum glutamic-oxalacetic transaminase. *J. Lab. & Clin. Med.*, 48: 144, 1956.
98. ROSALKI, S. B. and WILKINSON, J. H. A source of error in serum glutamic-oxaloacetic transaminase determination. *J. Clin. Path.*, 12: 138, 1959.
99. FRIEDEMANN, T. E. and HAUGEN, G. E. Pyruvic acid; determination of keto acids in blood and urine. *J. Biol. Chem.*, 147: 415, 1943.
100. TONHAZY, N. E., WHITE, N. G. and UMBREIT, W. W. A rapid method for the estimation of the glutamic-aspartic transaminase in tissues and its application to radiation sickness. *Arch. Biochem.*, 28: 36, 1950.
101. (a) CABAUD, P., LEEPER, R. and WROBLEWSKI, F. Colorimetric measurement of serum glutamic-oxaloacetic transaminase. *Am. J. Clin. Path.*, 26: 1101, 1956.
- (b) WROBLEWSKI, F. and CABAUD, P. Colorimetric measurement of serum glutamic pyruvic transaminase. *Am. J. Clin. Path.*, 27: 235, 1957.
102. REITMAN, S. and FRANKEL, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Path.*, 28: 56, 1957.
103. WROBLEWSKI, F. *Biochemical Biopsy via Body Fluids*. New York, 1958. Sloan-Kettering Institute for Cancer Research.
104. GUTTERIDGE, N. M. and MCKEAN, C. J. Serum transaminase; the diagnostic value of the estimation in cardiac infarction and liver damage. *M. J. Australia*, 1: 528, 1958.
105. LA DUE, J. S., WROBLEWSKI, F. and KARMEN, A. Serum glutamic-oxaloacetic transaminase activity in human acute transmural myocardial infarction. *Science*, 120: 497, 1954.
106. CHINSKY, M. and SHERRY, S. Serum transaminase as a diagnostic aid. *Arch. Int. Med.*, 99: 556, 1957.
107. BARON, D. N., BELL, J. and OAKLEY, C. Serum transaminase in coronary thrombosis and other conditions. *J. Clin. Path.*, 9: 389, 1956.
108. DERITIS, F., COLTORTI, K. and GUISTI, G. Diagnostic value and pathogenic significance of transaminase activity changes in viral hepatitis. *Minerva med.*, 47: 161, 1956.
109. WROBLEWSKI, F. *Advances in Clinical Chemistry*. Edited by Sobokta, H. and Stewart, C. P. New York, 1958. Academic Press.
110. CHINSKY, M., WOLFF, R. J. and SHERRY, S. Serum

transaminase activity: a comparison of the pyruvic and oxaloacetic transaminases. *Am. J. M. Sc.*, 233: 400, 1957.

111. HENRY, L. Serum transaminase levels in liver disease. *J. Clin. Path.*, 12: 131, 1959.
112. SHAY, H. and SIPLET, H. Study of chlorpromazine jaundice, its mechanism and prevention: special reference to serum alkaline phosphatase and glutamic-oxaloacetic transaminase. *Gastroenterology*, 32: 571, 1957.
113. SASS, M. and SPEAR, P. W. Whole blood transaminase levels in anaemia. *J. Lab. & Clin. Med.*, 51: 926, 1958.
114. PEARSON, C. M. Serum enzymes in muscular dystrophy and certain other muscular and neuromuscular diseases. I. Serum glutamic oxalacetic transaminase. *New England J. Med.*, 256: 1069, 1957.
115. LIEBERMAN, J., LASKY, I. I., DULKIN, S. I. and LOBSTEIN, O. E. Serum glutamic-oxalacetic transaminase activity in conditions associated with myocardial infarction. I. Bodily trauma. II. Cerebral vascular accidents and congestive heart failure. *Ann. Int. Med.*, 46: 485, 497, 1957.
116. NICKEL, W. K. and ALLBRITTEN, F. F., JR. Serum transaminase content related to tissue injury. *Surgery*, 42: 240, 1957.

Lactic dehydrogenase

117. WROBLEWSKI, F. and LADUE, J. S. Lactic dehydrogenase activity in blood. *Proc. Soc. Exper. Biol. & Med.*, 90: 210, 1955.
118. THIERS, R. E. and VALLEE, B. L. Enzymes in blood. *Ann. New York Acad. Sc.*, 75: 214, 1958.
119. MACDONALD, R. R., SIMPSON, J. R. and NOSSAR, E. Serum lactic dehydrogenase as a diagnostic aid in myocardial infarction. *J. A. M. A.*, 165: 35, 1957.
120. HANSON, A. and BJÖRCK, G. Glutamic-oxalacetic transaminase in the diagnosis of myocardial infarction. *Acta med. Scandinav.*, 47: 493, 1957.
121. WHITE, L. P. Serum enzymes. II. Glycolytic enzymes in patients with cancer and other diseases. *J. Nat. Cancer Inst.*, 21: 671, 1958.
122. WROBLEWSKI, F. Enzymes in blood. *Ann. New York Acad. Sc.*, 75: 322, 1958.
123. SCHAPIRA, G. and DREYFUS, J. C. Lacticodehydrase plasmatique au cours des myopathies. *Compt. rend. Soc. Biol.*, 151: 22, 1957.
124. (a) HILL, B. R. and LEVI, C. Elevation of a serum component in neoplastic disease. *Cancer Res.*, 14: 513, 1954.
- (b) HILL, J. H. Serum lactic dehydrogenase in cancer patients. *J. Nat. Cancer Inst.*, 18: 307, 1957.
125. WILSON, H. T., LAZARONI, J. A. and MAIER, E. C. A test for myocardial infarction. The present status of the use of serum lactic dehydrogenase. *California Med.*, 88: 369, 1958.
126. WHITE, L. P. Serum enzymes. III. Significance of abnormalities of glycolytic enzymes in the serum of cancer patients. *J. Nat. Cancer Inst.*, 21: 685, 1958.
127. HSIEH, K. M. and BLUMENTHAL, H. F. Serum lactic

dehydrogenase levels in various disease states. *Proc. Soc. Exper. Biol. & Med.*, 91: 626, 1956.

Malic dehydrogenase

128. WACKER, W. E. C., ULMER, D. D. and VALLEE, B. L. Metallo-enzymes and myocardial infarction. II. Malic and lactic dehydrogenase activities and zinc concentrations in serum. *New England J. Med.*, 255: 449, 1956.
129. SIEGAL, A. and BING, R. J. Plasma enzyme activity in myocardial infarction in dog and man. *Proc. Soc. Exper. Biol. & Med.*, 91: 604, 1956.
130. BING, R. J., CASTELLANOS, A. and SIEGAL, A. Diagnostic value of activity of malic dehydrogenase and phosphohexose isomerase. *J. A. M. A.*, 164: 647, 1957.

Isocitric dehydrogenase

131. WOLFSON, S. K., JR. and WILLIAMS-ASHMAN, H. G. Isocitric and 6-phosphogluconic dehydrogenases in human blood serum. *Proc. Soc. Exper. Biol. & Med.*, 96: 231, 1957.
132. WOLFSON, S. K., JR., SPENCER, J. A., STERKEL, R. L. and WILLIAMS-ASHMAN, H. G. Enzymes in blood. *Ann. New York Acad. Sc.*, 75: 260, 1958.

Glucose-6-phosphate dehydrogenase

133. ALTMAN, K. Erythrocyte glucose-6-phosphate dehydrogenase. *Am. J. Med.*, 27: 936, 1959.
134. LÖHR, G. W., WALLER, H. D., KARGES, O., SCHLEGEL, B. and MÜLLER, A. A. Zur Biochemie der Alterungsmenschlicher Erythrocyten. *Klin. Wchnschr.*, 36: 1008, 1958.

Glutathione reductase

135. MANSO, C. and WROBLEWSKI, F. Glutathione reductase activity in blood and body fluids. *J. Clin. Invest.*, 37: 214, 1958.

Choline esterase

136. MICHEL, H. O. Electrometric method for determination of red blood cell and plasma choline esterase activity. *J. Lab. & Clin. Med.*, 34: 1564, 1949.
137. ALDRIDGE, W. N. and DAVIES, D. R. Determination of choline esterase activity in human blood. *Brit. M. J.*, 1: 945, 1952.
138. REINHOLD, J. G., TOURIGNY, L. G. and YONAN, V. L. Measurement of serum cholinesterase activity by photometric indicator method. Together with study of influence of sex and race. *Am. J. Clin. Path.*, 23: 645, 1953.
139. SHIBATA, S. and TAKAHASHI, H. A simple procedure for the estimation of serum choline esterase by means of comparator with phenol red as indicator. *Bull. Yamaguchi M. School*, 1: 188, 1953.
140. BROWN, H. V. and BUSH, A. F. Parathion inhibition of cholinesterase. *Arch. Indust. Hyg.*, 1: 633, 1950.
141. CALLAWAY, S., DAVIES, D. R. and RUTLAND, J. P.

Blood cholinesterase levels and range of personal variation in a healthy adult population. *Brit. M. J.*, 2: 812, 1951.

142. HESTRIN, S. The reaction of acetyl cholin and other carboxylic acid derivatives with hydroxylamine and its analytical application. *J. Biol. Chem.*, 180: 240, 1949.
143. VORHAUS, L. J. and KARK, R. M. Serum cholinesterase in health and disease. *Am. J. Med.*, 14: 707, 1953.
144. MCARDLE, B. Cholinesterase in jaundice and diseases of the liver. *Quart. J. Med.*, 33: 107, 1940.
145. BARNES, J. M. and DAVIES, D. F. Blood cholinesterase levels in workers exposed to organo-phosphorus insecticides. *Brit. M. J.*, 2: 816, 1951.
146. LEHMANN, H. and RYAN, E. The familial incidence of low pseudo cholinesterase level. *Lancet*, 2: 124, 1956.

Ceruloplasmin

147. RAVIN, H. A. Rapid test for hepatolenticular degeneration. *Lancet*, 1: 726, 1956.
148. HOUGHIN, O. B. Rapid colorimetric method for quantitative determination of copper oxidase activity (ceruloplasmin). *Clin. Chem.*, 4: 519, 1958.
149. CUMINGS, J. N., GOODWIN, H. J. and EARLE, C. J. Blood copper and its relationship to the globulins. *J. Clin. Path.*, 8: 69, 1955.
150. THOMPSON, R. H. S. and CUMINGS, J. N. *Biochemical Disorders in Human Disease*. Edited by Thompson, R. H. S. and King, E. J. London, 1958. Churchill.
151. SCHEINBERG, J. H. and MORELL, A. G. Exchange of ceruloplasmin copper with ionic Cu^{64} with reference to Wilson's disease. *J. Clin. Invest.*, 36: 1193, 1957.
152. UZMAN, L. L., IBER, F. L., CHALMERS, T. C. and KNOWLTON, M. The mechanism of copper deposition in the liver in hepatolenticular degeneration (Wilson's disease). *Am. J. M. Sc.*, 231: 511, 1956.

REVIEWS

- Enzymes in blood. *Ann. New York Acad. Sc.*, vol. 75, 1958.
- KING, E. J. Enzymes in clinical biochemistry. *Clin. Chem.*, 3: 507, 1957.
- ABDERHALDEN, R. *Klinische Enzymologie. Die Fermente in der Pathogenese, Diagnostik und Therapie*. Stuttgart, 1958. Georg Thieme Verlag.
- GUTTERIDGE, B. H. Enzymes in clinical medicine. I. *Therapeutica Nova*, 11: 3, 1958.
- ALLDIS, D. Enzymes in clinical medicine. II. *Therapeutica Nova*, in press.
- BODANSKY, O. Blood biochemical alterations in neoplastic disease. *M. Clin. North America*, 40: 611, 1956.
- GOMORI, G. Methods for quantitative estimation of hydrolytic enzymes in serum and other biologic fluids. *Am. J. Clin. Path.*, 24: 99, 1954.
- WROBLEWSKI, F. *Biochemical Biopsy via Body Fluids*. New York, 1958. Sloan-Kettering Institute.

Symposium on Diagnostic Enzymology

Diagnostic Applications of Enzymes in Medicine*

General Enzymological Aspects

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THE rapidly expanding role of enzymes in elucidating the steps in the intricate sequences of normal and abnormal metabolism has constituted one of the most impressive advances in biochemistry during the past three decades. All fields of medicine have felt the impact of these advances. It seems appropriate, in connection with the present symposium on the diagnostic applications of enzymes in medicine, to summarize some of the basic properties of enzymes and the general characteristics of their actions.

Enzymes are protein catalysts. As such, they increase greatly the rate of chemical reactions involved in various metabolic sequences, and therefore make possible the various functions characteristic of living matter. Enzymes are effective in small amounts, are usually specific with respect to the type of chemical reaction that they mediate and, except for some occasional denaturation, do not change in concentration during the course of the reaction. About 700 enzymes have been identified in human, animal, plant and microbial organisms. By 1956 approximately seventy-five enzymes had been isolated as crystalline proteins [1].

ENZYMES AS PROTEINS

Enzyme Size and Structure. The usual methods for determination of the molecular weights of proteins have been applied to enzymes. Although some degree of uncertainty attends several of the values assigned, the following are illustrative of current estimates: ribonuclease, 12,700; pepsin, 34,500; pancreatic α -amylase,

45,000; phosphoglucomutase, 74,000; yeast lactate dehydrogenase, 100,000; muscle aldolase 147,000 to 180,000; heart fumarase, 204,000; soy bean urease, 480,000; L-glutamate dehydrogenase, 1,000,000 [1].

Determination of the terminal α -amino groups by the dinitrofluorobenzene method of Sanger [2,3] and of the terminal carboxyl groups by the action of carboxypeptidase has indicated that many of the enzymes which have so far been studied, for example, ribonuclease, lysozyme, papain, trypsin and pepsin, have only a single peptide chain. Rabbit muscle aldolase and α -chymotrypsin have been shown to have two peptide chains. Most enzymes are globular proteins.

The methods developed by Sanger for determining the amino acid sequence in proteins have been applied to several enzymes. Naturally, those of lower molecular weight, such as ribonuclease (12,700), lysozyme (17,000) and papain (20,700), have been the first subjects of study. Approximately 125 amino acids have been found in the single chain of ribonuclease and the sequence in which they appear has been fairly well determined [4,5]. Similar studies have been carried out with lysozyme, papain and even some of the larger enzymes.

Active Center of the Enzyme. The active center may be defined as the particular set of chemical groups in the protein enzyme molecule that combine specifically with the substrate molecule and are responsible for the characteristic catalytic reaction, and may be conceived as lying in one peptide chain or across adjacent

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chains. The chemical nature of the active center has been studied by deducing the pK values from the effect of pH on the substrate-enzyme combination, by degradation studies of the protein molecule, and by inactivation of the enzyme by specific chemical reagents.

These methods may be briefly illustrated. In enzymes that have a pK of about 6 to 7, the assumption may be tentatively made that the active center contains amino acids, such as histidine, with a pK in this region. In ribonuclease the sequence of the last four amino acids at the C-terminal end is: -aspartic-alanine-serine-valine. Carboxypeptidase removes the last three of these without loss of activity. Pepsin removes the four amino acids as a tetrapeptide, which renders the remainder of the molecule inactive. Therefore, although the aspartic acid residue would appear to be part of the active center, its presence is a necessary but not a sufficient condition for ribonuclease activity. Oxidation of the disulfide bridge between different parts of the molecule results in inactivation that may be interpreted as a separation of parallel sections of the folded chain and disruption of the constellation of amino acid residues that constitute the active center [6]. In papain, the action of aminopeptidase can remove 120 of the 180 amino acid residues from the N-terminal end without altering the original activity with respect to the hydrolysis of benzoylarginine-amide [7].

Thiol-alkylating agents such as ethyl iodacetate or chloracetophenone, mercaptide-forming reagents such as p-chloromercuribenzoate or trivalent arsenicals, and thiol-oxidizing agents such as iodosobenzoate markedly inhibit several classes of enzymes, indicating that the sulfhydryl group is an important group in the active center of these enzymes. Certain enzymes, for example, cholinesterase, trypsin or chymotrypsin, are rendered inactive by combination with organophosphorus compounds, such as diisopropyl-fluorophosphate (DFP), which apparently combine with serine in an amino acid sequence that is part of the active center of the enzyme. The presence of a phosphate group, probably at a serine residue, is essential for the action of phosphoglucomutase [8].

CLASSIFICATION OF ENZYMES

Enzymes may be classified in either of two major ways, namely, by the type of chemical action on the substrate, or by the sequence of enzymes necessary to accomplish certain important metabolic actions.

Classification by Action on Substrate. Most schemes of classification have been based on the nature of the chemical bond that is acted upon by the enzyme. Although many of the classifications differ in details, certain general features are common to most of them. One of the more convenient classifications [9] divides enzymes into the following major groups. The *hydrolytic enzymes* cleave the substrate by the introduction of water. Thus esterases split an acid-alcohol bond and include such subgroups as lipase and the phosphatases and nucleases. Carbohydrases cleave glucosidic linkages and proteases the C-N linkage. *Phosphorylases* catalyze phosphorylytic cleavage of the α -glucosidic-1,4-linkages of polysaccharides. The class of *oxidation-reduction enzymes* includes the dehydrogenases that utilize coenzymes, the aerobic dehydrogenases, the flavin enzymes, and oxidases such as tyrosinase and catalase. In the class of *transferases* are to be found such enzymes as transaminases and phosphokinases. The *decarboxylases* mediate the removal of CO₂ whereas the *hydrases*, as exemplified by fumarase and enolase, are involved in the reversible addition of water. The *isomerases* and *mutases* mediate intramolecular arrangements as, for example, the reversible conversion of glucose-6-phosphate to fructose-6-phosphate.

Classification by Metabolic Sequence. Enzymes may also be classified by the sequence necessary to accomplish certain important metabolic actions. Although these various sequences are interlinked and "feeder" lines may branch out from one sequence to merge with another, certain major paths are discernible. Thus the glycolytic system, whereby glucose is converted to lactate, consists of thirteen consecutive enzyme-mediated reactions [7]. The citric acid cycle is initiated by the oxaloacetate transacetylase-mediated combination of acetyl-coenzyme A and oxaloacetic acid, then passes through a series of reactions catalyzed by aconitase, isocitric dehydrogenase, ketoglutaric dehydrogenase, thiol transacylase, succinyl-CoA deacylase, succinic dehydrogenase, fumarase and malic dehydrogenase to yield oxaloacetic acid again. The glucose-6-phosphate oxidation sequence, the phenylalanine oxidation system, and the "urea cycle" constitute other examples of major metabolic pathways.

ENZYME COFACTORS

Many enzyme-catalyzed reactions require, in addition to substrate and enzyme, some other substance or "cofactor" in relatively low con-

centrations. The term "activator" is usually applied to metal ions or small non-specific organic molecules, whereas the term "coenzyme" is reserved for specific organic molecules which play a part in the reaction itself, very often as carriers of some chemical grouping. When the coenzyme is firmly attached to the enzyme protein it is designated a "prosthetic group."

Enzyme Activators. Metal cations: The following cations have been found to act as activators of one or more enzymes: NH_4^+ , Na^+ , K^+ , Rb^+ , Cs^+ , Mg^{++} , Ca^{++} , Zn^{++} , Cd^{++} , Cr^{+++} , Cu^{++} , Mn^{++} , Fe^{++} , Co^{++} , Ni^{++} , Al^{+++} . These activations may be grouped as follows: enzymes for which only one of these ions is capable of acting as activators as, for example, Mg^{++} in the case of 5-nucleotidase or Mn^{++} in the case of imido-dipeptidase; enzymes for which any of several ions may serve as activators, such as Mg^{++} , Mn^{++} or Zn^{++} for enolase; enzymes that require a combination of more than one ion, such as Mg^{++} and K^+ , Rb^+ or Cs^+ for pyruvate phosphokinase. Some of the ions listed can also antagonize the activating actions of others. For example, Na^+ or Li^+ counteracts the activating action of K^+ or NH_4^+ on phosphotransacetylase and Ca^{++} antagonizes the activating effect of Mg^{++} or Mn^{++} on adenosine triphosphatase [1,10].

If the concentration of an activating metal ion is increased, the reaction velocity increases, reaches an optimum, and then decreases at higher concentration of metal ion. Study of this relationship at various concentrations of substrate has led to several formulations of the mechanism of metal ion activation. The metal may (1) form an essential part of the active enzyme center; (2) interact with enzyme and substrate to form an active complex of all three; (3) form an active complex with the enzyme to which the substrate can more readily attach itself; (4) interact with the apparent substrate to yield a complex that acts as the real substrate for the enzyme; (5) change the equilibrium constant of the enzyme reaction; (6) exert an indirect accelerating action, such as combining with an inhibitor present in the enzyme preparation or displacing ineffective metal ions from combination with the active center of the enzyme. The assumptions that have just been noted form the basis for mathematical formulation that account, to varying degrees, for the kinetics of the enzyme reactions in the presence of these metal activators [11-13].

Anions: Several enzymes are activated by the presence of anion, usually however at appreciable concentrations of about 0.1 M to 0.01 M. The activation of α -amylase by chloride and other anions, and of fumarase by arsenate, phosphate, citrate or selenate illustrate these effects. Changes in the pH-optimum may also occur. These effects, explained by changes in the ionization of the intermediate enzyme-substrate complex, are of practical importance since the anions may be present in buffer solutions, and the choice of different buffers may influence the results obtained in kinetic studies.

Non-specific organic compounds: A number of enzymes are activated by organic compounds in a non-specific manner. For example, the proteinase, ficin, is activated by thioglycollate as well as by cyanide. Very low concentrations of amino acids activate alkaline phosphatase and other enzymes [14]. Although the possibility exists that, in such instances, activation may be due to complexing of the amino acids with inhibiting heavy metal ions present as impurities, cysteine, histidine and other amino acids have been found necessary for the action of crystalline phosphoglucomutase [15].

Specific Coenzymes. General considerations: Coenzymes are organic compounds which, in the presence of specific enzymes, are capable of transferring chemical groups to or, reversibly, accepting them from various substrates. The general formulation of the function would be:

$$S + \text{CoX} \xrightleftharpoons{E_1} \text{SX} + \text{Co}$$
 where S designates the substrate, and X and Co are the chemical group and coenzyme, respectively. It is apparent, first, that a coenzyme may be present in two forms, i.e., acceptor and donator, and secondly, that in reactions of the type noted, the coenzyme reacts stoichiometrically and may, in a sense, be also regarded as a substrate.

The reaction, $S + \text{CoX} \xrightleftharpoons{E_1} \text{SX} + \text{Co}$, may be linked directly with another enzyme reaction in which the coenzyme partakes as follows: $\text{TX} + \text{Co} \xrightleftharpoons{E_2} \text{T} + \text{CoX}$, where TX is a substrate from which the coenzyme, Co, is capable of accepting the chemical group, X. Thus the coenzyme is regenerated in a form in which it may again be used in the first reaction.

In some reactions or sequences of reactions the coenzyme does not clearly appear in two forms, but as an intermediate through which the substrate passes to form its reaction product. For

example, according to recent investigations [16] the substrate, glucose-1-phosphate reacts with the active, phosphorylated form of the enzyme, phosphoglucomutase, to form the coenzyme, α -glucose-1,6-diphosphate, and the inactive, dephosphorylated enzyme. These two then interact in a second, successive reaction to form the reaction product, glucose-6-phosphate, and to regenerate the active phosphorylated enzyme.

Hydrogen carriers: The common hydrogen carriers are, as expressed in their oxidized forms: coenzyme I or diphosphopyridine nucleotide (DPN), coenzyme II or triphosphopyridine nucleotide (TPN), lipoic acid, glutathione, and the cytochromes. The structural formulas of these compounds may be found in standard biochemistry texts. The structure of coenzyme I (DPN), for example, consists of the nucleotide, adenosine-5'-phosphate linked to the 5-phosphate of nicotinamide-D-riboside. Reduction or acceptance of hydrogen takes place at the 1:4 positions of the nicotinamide ring. A similar reduction takes place in coenzyme II or triphosphopyridine nucleotide (TPN). Some of the enzyme-mediated reactions associated with the reduction of DPN to DPNH₂ are the oxidation of substrates containing the following structure: $-\text{CHNH}_2\cdot\text{COOH}$ to $-\text{CO}\cdot\text{COOH}$ and of $-\text{CH}\cdot\text{OH}\cdot\text{COOH}$ to $-\text{CO}\cdot\text{COOH}$. In some of these reactions TPN rather than DPN acts as the coenzyme.

Lipoic acid, in its oxidized form, has a disulfide group which may be reduced to two thiol groups. In the presence of pyrophosphothiamine and the specific dehydrogenase, oxidized lipoate reacts with pyruvate or α -ketoglutarate to form, respectively, 6-S-acetylhydrolipoate and CO₂, or 6-S-succinylhydrolipoate and CO₂. The reduction of the lipoate is also accompanied by a transfer of the acyl group. **Glutathione** is a tripeptide of cysteine, glutamic acid and glycine, and in its oxidized form consists of two molecules linked through a disulfide group: $\text{GSSG} + 2\text{H} \rightleftharpoons 2\text{GSH}$. It acts as a coenzyme for the glyoxalase system, for formaldehyde dehydrogenase, and for the conversion of 4-maleylacetoacetate to 4-fumarylacetoacetate in the presence of maleylacetoacetate isomerase. The **cytochromes** are intracellular proteins which are combined with distinctive porphyrins that exist in reduced and oxidized forms, readily convertible into each other. For example, reduced cytochrome C reacts with molecular oxygen in

the presence of cytochrome oxidase to form oxidized cytochrome C and water.

Phosphate carriers: Another biologically important carrier-donor system is adenosinediphosphate-adenosinetriphosphate. There is some evidence that di- and triphosphates of other nucleosides may also act in this manner. A large number of phosphokinases mediate the transfer of a phosphate group from adenosine triphosphate (ATP) to various monosaccharides, monosaccharide derivatives, purines and pyrimidines, to yield the phosphorylated compounds and adenosine diphosphate (ADP). On the other hand, phosphoglycerate kinase mediates the acceptance of a phosphate group by adenosine diphosphate (ADP) from D-1,3-diphosphoglyceric acid to form ATP and D-3-phosphoglyceric acid. Pyruvate phosphokinase similarly mediates the interaction of phosphoenolpyruvic acid and ADP to form pyruvic acid and ATP.

Acyl-group carriers: Coenzyme A is 3'-phospho-ADP-pantoyl- β -alanyl-mercaptoethanolamine. The thiol group of the mercaptoethanolamine is the functional part of the coenzyme. In the presence of various syntheses and ATP, coenzyme A can combine through the $-\text{SH}$ group with acetate, fatty acids and other substances to form the corresponding coenzyme A derivatives. Conversely, there are many transacylases that can transfer the group linked to coenzyme A to other compounds. For example, oxaloacetate transacetase catalyzes the reaction: $\text{Acetyl-CoA} + \text{oxaloacetate} \rightleftharpoons \text{CoA} + \text{citrate}$.

Other coenzymes: Uridine diphosphate glucose interacts with α -galactose-1-phosphate in the presence of uridyl transferase to form uridine diphosphate galactose and α -glucose-1-phosphate. The action of the coenzyme, α -glucose-1-6-diphosphate, has previously been described. In the presence of the specific enzyme, the folic acid derivative, tetrahydrofolate, is capable of taking up the hydroxymethyl group, $-\text{CH}_2\text{OH}$, from serine or the formimino group, $-\text{CH}-\text{NH}-$, from formiminoglycine or formiminoglutamate. The groups which are thus added are then handed on in other enzymic reactions.

Prosthetic Groups. It was noted at the beginning of this section that a compound possessing all the characteristics of a coenzyme may be bound firmly to the enzyme protein and is then designated a prosthetic group. A number of flavoprotein enzymes such as D-amino acid oxidase, xanthine oxidase, cytochrome C reduc-

tase contain *flavin adenine dinucleotide (FAD)* or *flavin mononucleotide (FMN)* as prosthetic groups. Reduction or oxidation involves the isoalloxazine nucleus in these groups, reduction consisting of the addition of two hydrogen atoms across the quinonoid structure. *Phosphopyridoxal* is the prosthetic group in several enzymes which catalyze a variety of reactions involving amino acids, such as the transaminases, and amino acid dehydrases and racemases. *Pyrophosphothiamine* (thiamine pyrophosphate) is the prosthetic group of several enzymes, chiefly those catalyzing the decarboxylation of α -keto acids.

Vitamins as Enzyme Cofactors. As will have been noted, many of the coenzymes and prosthetic groups contain, as part of their structure, the molecular grouping of a vitamin or closely related derivative. Thus, of the members of the vitamin B group, nicotinamide is present in coenzyme I (DPN) and coenzyme II (TPN), pantothenic acid in coenzyme A, riboflavin in the prosthetic mono- and dinucleotides, thiamine in pyrophosphothiamine, and pyridoxine (vitamin B₆) in phosphopyridoxal.

Several vitamins or closely related derivatives act directly as coenzymes or partake in interactions with coenzymes. Ascorbic acid, the antiscorbutic vitamin, serves as a hydrogen carrier. There is also some evidence that vitamins E and K act in a similar fashion. The coenzyme, tetrahydrofolic acid, is the vitamin, folic acid, with the pteridine nucleus in the reduced tetrahydro form. Vitamin A₁, necessary for vision in dim light, interacts reversibly with DPN in the presence of an alcohol dehydrogenase to form DPNH and vitamin A₁ aldehyde (retinene₁).

The role of vitamins as coenzymes or prosthetic groups has several features of biological interest. By definition a vitamin is a substance which cannot be synthesized by the organism, at least at a rate sufficient for needs, and, unless formed by the organism's intestinal flora, must be present in the diet to assure normal biochemical and physiological functions. Absence of vitamins from the diet should therefore result in inadequate activity of enzymes requiring these vitamins or their derivatives as coenzymes and prosthetic groups. Unless alternate metabolic pathways are invoked, metabolic derangements should follow. A number of such instances have been noted. The elevated blood pyruvic acid in thiamine deficiency may be explained by a decrease in the content of the coenzyme,

pyrophosphothiamine, and the activity of the corresponding enzymes necessary for the metabolic conversions of pyruvic acid. D-amino acid oxidase and xanthine oxidase are both flavoprotein enzymes, and it has been reported that the content of these enzymes is decreased in the liver of riboflavin-deficient rats. However, it should be stressed that many of the characteristic changes of vitamin inadequacies, such as the atrophy of epithelial cells in vitamin A deficiency, the neuritis and cardiovascular changes of thiamine deprivation, or the cheilosis and nasolabial seborrheic dermatitis of human riboflavin deficiency cannot as yet be explained in terms of altered enzymic activities and deranged metabolic pathways.

Compounds closely related in structure to various vitamins with coenzyme or prosthetic group function may interact with the corresponding enzymes in place of the normal vitamins. If these related compounds do not contain the chemical groupings essential for coenzyme activity, they will tend to counteract the role of the specific vitamin and to lead to metabolic derangements and avitaminotic states. Indeed, such compounds have been termed "antivitamins." For example, aminopterin, the compound resulting when an amino group replaces the hydroxyl group in folic acid, antagonizes the action of tetrahydrofolate, and pyridine-3-sulfonic acid neutralizes the action of nicotinic acid.

FACTORS INFLUENCING ENZYME ACTION

Measurement of Reaction Velocity. Conceptual considerations: Before attempting any evaluation of the factors which may affect the rate of an enzyme catalyzed reaction it is essential that properly conceived measures of reaction velocity be employed [17]. The relation between a reaction constant, k , the concentration of reactant changed, x , and the time, t , necessary for this change may be generally expressed as

$$t = \frac{1}{k} \times f(x) \quad (1)$$

where x is a continuous function of t . If it is assumed that the form of the function does not change as a given condition is varied, then it can be shown that for any two variants of this condition, and for a given value of $t = t_1$

$$k_A/k_B = f(x_A)/f(x_B) \quad (2)$$

and for a given value of $x = x_1$

$$k_A/k_B = (1/t_A)/(1/t_B) \quad (3)$$

Three types of measures of reaction velocity may therefore properly be used: (1) reaction constants expressing the entire course of a reaction, (2) the reciprocals of the times necessary to effect a given change, (3) ratio of the *functions* of the amounts changed in a given time. Monomolecular or other types of constants formerly were used as measures of reaction velocity, but such "constants" applied only to specialized conditions and frequently changed during the course of the reaction. Their use violated the postulates implicit in equations 1 to 3 and frequently introduced large errors into the evaluation of factors influencing enzyme activity [17]. The second type of measure of reaction velocity, namely, the reciprocal of the time necessary to effect a given change may validly be used when the form of the time-change curve, although unknown, does not change with different variants of a given condition. Such constancy is assured if the ratio of the reciprocals of the time required to effect a given change has the same value for any change in the course of the time-change function. This type of measure of reaction velocity, although formerly frequently employed, has been largely discarded in current enzymologic research.

The most commonly used measure of reaction velocity at present is that derivable from equation 2, namely the function of the amount changed in a given time. In a reaction of zero order, $x = kt$, the function of the amount changed in a given time is identical with the amount itself. The activity of an enzyme may therefore properly be measured by determining the amount of substrate changed in a stated time during the initial zero order portion of the reaction. Attention to experimental conditions is necessary so that the changes of the substrate that are measured do indeed fall within the initial portion of the reaction.

Since most enzyme reactions are of zero order only during the first 5 to 10 per cent change of the substrate, the use of this measure involves a considerable wastage of substrate. When the substrate is expensive or scarce and only low concentrations can be used, it is possible to utilize a fourth measure of reaction velocity in which the amount of substrate changed *at any stage of the reaction* may properly be employed for the measurement and comparison of enzyme

activities [18]. This measure is based on setting up a reference curve showing the changes in substrate in a given time, T , at various concentrations of a reference enzyme preparation.

Measurement in tissue enzyme preparations and in serum: Obviously the isolation of the crystalline enzyme from a tissue and measurement of its activity, although theoretically desirable, would involve losses of enzyme and be so laborious and time-consuming as to make this procedure utterly impracticable. Instead, three general types of preparations are used—tissue slices, homogenates, and aqueous or other extracts. Reaction conditions and the measure of reaction velocity are chosen so that measurement of the enzyme activity is a precise, quantitative reflection of the concentration of enzyme in such preparations. The time between removal of the tissue from the organism and the measurement of activity is made as short as possible in order to minimize the processes, themselves frequently enzymic, that tend to destroy the enzymes. Refrigeration at 0° to 4°C. for several hours or in the deepfreeze at -15° to -20°C. for periods of several days is effective, but the stability of each enzyme under these conditions should be determined.

Tissue slices are used when it is desired to approximate the metabolic activity of the tissue *in vivo*. Homogenization of tissue is generally accomplished by various types of blenders or of apparatus consisting of a test tube and close-fitting power-driven pestle. Usually, cell disruption can be made fairly complete, and the destruction of nuclei, mitochondria and other particulates is minimal. Several kinds of media, such as water, isotonic sodium chloride solution, various types of Ringer solutions and isotonic (0.25 M) sucrose may be employed for the purpose; the choice depends upon the degree to which it is desired to keep the cell particulates intact. The third chief procedure for obtaining enzymes from tissue is extraction. Many enzymes, like other proteins, are soluble in dilute salt solutions at pH values other than their isoelectric point and may thus be removed from finely minced or homogenized tissues. For other enzymes, physical methods or organic solvents may be necessary for more complete extraction.

Enzymes in serum or other body fluids do not pose any problem of extraction, and have been used widely in diagnostic biochemistry. The chief practical concern in this connection is the

stability of these enzymes. Blood samples drawn for enzyme and other biochemical determinations are at room temperature for several hours during transportation to and processing in the laboratory. Serum glutamic oxaloacetic and pyruvic transaminases, lactic dehydrogenase and phosphohexose isomerase are stable for at least eight hours at room temperature. The rapid loss of serum glucose-6-phosphate dehydrogenase and other enzymes over a period of one to five hours at room temperature makes their diagnostic application difficult. The stability at refrigerator or deepfreeze temperature varies widely for different serum enzymes. Phosphohexose isomerase is stable for at least four weeks at refrigerator temperature and for at least a year in the deepfreeze. Serum glutamic oxaloacetic transaminase at normal activities is stable for at least ten months, and at high activities for approximately four months. These few examples emphasize the necessity for determining the stability of serum enzymes before determinations of their activity are employed in diagnosis.

Inhibitors and activators in enzyme preparations: Determination of the enzyme activities of serum, other body fluids, tissue homogenates, or tissue extracts should be undertaken only when there is assurance that a plot of the reaction velocity, properly measured, against the concentrations of serum and tissue homogenate, to be used in the assays, yields a straight line relationship. A curve that is "concave upwards" usually indicates suboptimal concentrations of necessary activators in the enzyme preparation, and a "concave downwards" curve indicates the presence of inhibiting substances. The addition of the necessary activators in optimal concentration or the removal of inhibitors by a simple procedure such as dialysis, or measurement under conditions in which the inhibitors have no effect will establish a straight line relationship and thus give assurance that the reaction velocity is indeed a measure of the concentration of the enzyme protein rather than of other factors influencing its activity.

Enzyme Concentration. The rate of action of an enzyme is directly proportional to its concentration. Although several exceptions to this law have apparently been noted, closer examination reveals that these exceptions are due either to the use of incorrect measures of reaction velocity or to adventitious factors which influence the activity of the enzyme.

Deviation from linear relationship due to incorrect measure of reaction velocity: It has been stated in the past and is still repeated in some monographs and texts [19,20] that for certain proteolytic enzymes the activity is proportional to the square root of the concentration of enzyme, the so-called Schütz law. This formulation is based on the use of an incorrect measure of reaction velocity, and it has been shown mathematically from Schütz' own data [21] that the reaction velocity is directly proportional to the concentration of enzyme, not to the square root thereof [22]. In studying the velocity-enzyme concentration relationship by means of initial reaction velocities there must be assurance that the concentration of substrate is sufficiently high so that even at the very highest concentration of enzyme used the initial velocities measured are within the zero order portion of the reaction.

Deviations from linear relationship due to technical factors: In manometric measurements the uptake of oxygen or the liberation of CO₂ is dependent upon the rate of diffusion of gas into the liquid and this, in turn, may be dependent upon the rate of shaking. At higher concentrations of tissue slices or homogenates the increased liberation or utilization of gas may not be manifest, and the velocity-enzyme concentration curve will be concave-downwards in form. Increasing the rate of shaking restores the relationship to a linear one.

When the velocity of one enzyme reaction is measured by the rate at which its reaction product is disposed of by a second enzyme reaction, $A \xrightarrow{E_1} B \xrightarrow{E_2} C$, the second enzyme must be in excess at all concentrations of E₁. Otherwise, at higher concentrations of E₁ the manifest rates of reaction are lower than they should be, and the velocity-enzyme concentration relationship apparently departs from linearity.

Another deviation from linearity may appear to exist in complex enzyme systems, as has been reported by Straub [23]. Lactic dehydrogenase and diaphorase (flavoprotein) catalyze the following sequence: lactic acid + coenzyme 1 \rightleftharpoons pyruvic acid + reduced coenzyme 1; reduced coenzyme 1 + FAD \rightleftharpoons coenzyme 1 + reduced FAD; reduced FAD + methylene blue \rightleftharpoons FAD + leucomethylene blue. With a small, constant amount of coenzyme and a high concentration of flavoprotein, an increase in the concentration of lactic dehydrogenase first caused a rise and then a decrease in the reaction velocity, as

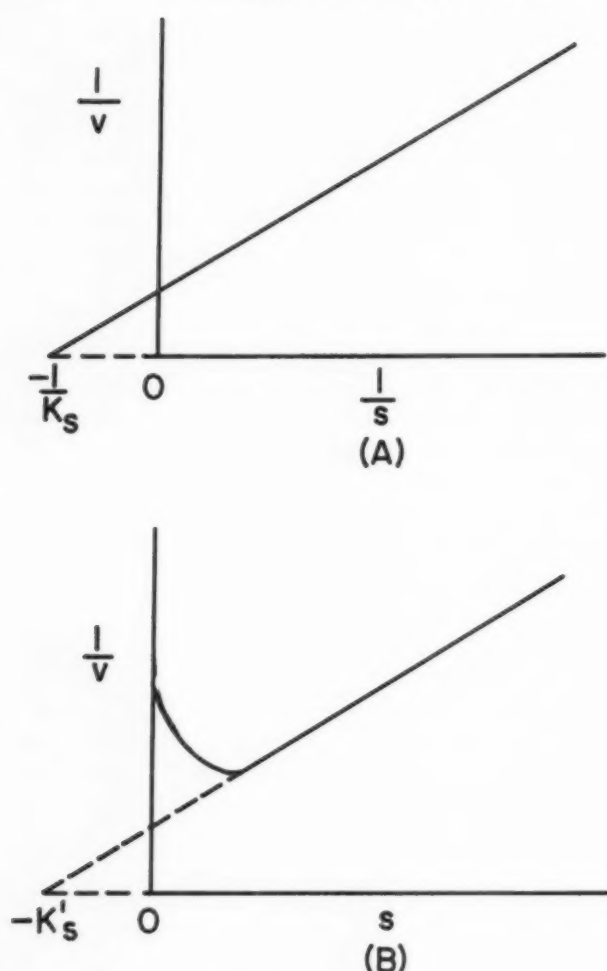


FIG. 1. Relationship between velocity and substrate concentration. A, plot of reciprocal of reaction velocity against reciprocal of substrate concentration to yield value of K_s . B, plot of reciprocal reaction velocity against normal and excess substrate concentrations to yield value of K'_s .

measured by the reaction of leucomethylene blue with atmospheric oxygen. With further increase in concentration of lactic dehydrogenase the reaction velocity decreased to negligible levels. This was explained by a combination of the dehydrogenase and coenzyme 1 in the first reaction to such an extent that coenzyme was not available for the subsequent reactions.

Influence of inhibitors and activators: These effects have been explained in a previous section, and their description need not be repeated here, except for one interesting instance. With purified or crystalline preparations, the reaction velocity may not increase or may not increase proportionately at low concentrations of enzyme; beyond this "lag" period, however, a linear relationship holds. The lag period is due to

the presence of impurities in the water or reagents of the reaction mixture which combine with and render a small portion of the enzyme inactive.

Substrate Concentration. When the concentration of substrate is increased, other conditions being held constant, the reaction velocity increases—rapidly at low concentrations, more slowly at higher concentrations. In general, the form of this increase resembles a rectangular hyperbola. For many enzymes, a point of saturation is reached so that further increases in the concentration of substrate do not result in any further increase in velocity. However, for some other enzymes increases in the concentration of substrate result in a decrease of reaction velocity, sometimes to an exceedingly small fraction of the maximal velocity. More complex relationships, such as the simultaneous action of one enzyme or two substrates or of two or more enzymes on the same substrate, have also been formulated but will not be discussed in this review.

The Michaelis-Menten formulation: The assumptions underlying this formulation [24] are contained in the following equations: $E + S \rightleftharpoons ES$; $ES \rightarrow E + \text{products}$. The enzyme forms an intermediate complex, ES. Equilibrium is attained so rapidly in comparison with the breakdown of ES that ES remains always in equilibrium with E and S while the enzyme action is proceeding. The reaction velocity is proportional to the concentration of ES. These assumptions lead to the following expression:

$$\frac{v}{V_{max}} = \frac{s}{s + K_s} \quad (4)$$

This equation accounts fairly well for data obtained in those enzyme actions in which the velocity attains a limiting maximal value at very high concentrations of substrate. v is the velocity at any concentration of substrate, s , and V_{max} is the theoretic maximal velocity. K_s is the dissociation constant of the intermediate enzyme compound and, as may be seen from equation 4, is equal to that concentration of substrate, s , at which v is equal to one-half the value of V_{max} .

Variants of the Michaelis-Menten formulation: Equation 4 may be transposed algebraically into several other forms, of which the most common is the Lineweaver-Burk transposition [25]. The reciprocal of the velocity is plotted as

ordinate against the reciprocal of the substrate as abscissa. (Fig. 1A):

$$\frac{1}{v} = \frac{1}{s} \times \frac{K_s}{V_{\max}} + \frac{1}{V_{\max}} \quad (5)$$

At zero value for the abscissa, $\frac{1}{s} = 0$, the intercept on the axis of ordinates yields the value for the maximal velocity, $\frac{1}{v} = \frac{1}{V_{\max}}$. At zero value

for the ordinate, $\frac{1}{v} = 0$, the intercept on the axis of abscissae gives the value for the dissociation constant of the intermediate complex, $1/K_s = -1/s$ or $K_s = -s$. The terms K_s and K_m have frequently been used interchangeably. Dixon and Webb [7] have suggested that " K_s " be used to designate the dissociation constant of the enzyme-substrate complex whereas " K_m " or the "Michaelis constant" be restricted to designate the substrate concentration giving half maximal velocity. Although these two constants are identical in the simple Michaelis theory as set forth herein, they may yield different values for enzymes possessing a very high catalytic activity [7].

Inhibition by excess substrate concentration: High concentrations of substrate may decrease the reaction velocity by decreasing the concentration of water in which the reaction is taking place, by combining with an activator essential for the reaction, or because of crowding of substrate molecules on the enzyme, combining at only one of two or more necessary areas in the active centers and thus forming enzymically inactive complexes: $E + S \rightleftharpoons ES$ (active); $ES + S \rightleftharpoons ES_2$ (inactive). These assumptions lead to the formulation of the following expression [25]:

$$\frac{1}{v} = \frac{1}{V} + \frac{s}{K'_s V} \quad (6)$$

where v is the velocity at substrate concentration, s , and V is the velocity that would be obtained if the reaction velocity did not decrease at higher concentrations of substrate. When values of $\frac{1}{v}$ are plotted as ordinates against values of s as abscissae, a curvilinear relationship at low concentrations of substrate merges into a linear relationship at high concentrations of substrate. (Fig. 1B.) The intercept of the straight line at $s = 0$ yields the value of V , and extra-

polarization to the axis of abscissae at $\frac{1}{v} = 0$ yields the value for $K'_s = -s$.

Effect of pH. The activity of an enzyme may be influenced by pH by irreversible destruction at points on either side of the optimum, or by the effects on the ionization of the enzyme, the substrate or the enzyme-substrate complex. It has long been appreciated that when an enzyme is exposed for varying periods of time at pH levels removed from the optimum and is then tested at the optimum, the activity is less than that obtained before exposure. For example, the optimal pH of yeast invertase is about 4.7, but this enzyme undergoes inactivation at pH levels below 2.5 at 22°, 3.0 at 30° and 3.9 at 52°C. [26]. Indeed, when a pH velocity curve is determined for any enzyme it is necessary to have assurance that inactivation due to pH does not occur during the period used for the determination of reaction velocities. When this factor of irreversible inactivation is taken into account the pH-velocity relationship is usually such that the velocities rise from values at low pH's to a plateau-like optimum, then fall away again at alkaline pH values.

The effect of pH has been treated generally by a number of investigators, particularly by Laidler [27]. Hase [28] observed that, for the action of equine serum cholinesterase on acetylcholine, the Michaelis constant ranged from a log value of about -1.3 at pH 5.0 to one of about -4.0 at pH levels beyond about 8.0. Bull and Currie [29] found that V_{\max} for the digestion of egg albumin by crystalline pepsin at 30°C. decreased from a value of 0.092 at pH 1.34 to 0.013 at pH 2.80. The Michaelis-Menten constant increased from 0.67×10^{-4} at pH 1.34 to 7.35×10^{-4} at pH 2.80. The latter results were consistent with the formulation that the intermediate complex combines with H^+ to form an activated complex $[H^+ ES]$ and that this complex breaks down to form the reaction product, P , and regenerate the free enzyme, E .

Effect of Temperature. Increasing the temperature of an enzyme reaction has two main effects—first, heat inactivation of the enzyme protein and, secondly, increase in the rate of reaction in accordance with the Arrhenius formulation. The first of these effects may be evaluated by determining the activity before and after incubation of the enzyme at various temperatures for varying periods of time. This is accomplished by composing an incubation mixture

containing enzyme, buffer and necessary activators; samples taken from this mixture at various times are added to substrate, and the initial reaction velocity is determined. A decrease in the initial reaction velocity in a sample indicates the time at which inactivation of the enzyme has begun at the stated temperature. Indeed, studies of this type should be carried out before the influence of temperature on reaction rates is considered, in order to assure that the observed reaction rates are not complicated by inactivation of the enzyme.

Heat inactivation of enzymes: Inactivation of enzymes by heat is due to denaturation of the enzyme protein. The extent of inactivation depends upon both the temperature and the length of exposure and varies widely for different enzymes. Many enzymes in purified or crystalline form are inactivated within a few minutes at temperatures of 60° to 80°C. but there are both less and more resistant enzymes. The susceptibility to heat may also be expressed by the values for activation energy for inactivation; these are characteristically high and range from 40,000 to 100,000 calories per mole [30].

Influence on rate of reaction: It is frequently stated that a rise of 10° increases the rate of an enzyme reaction two- to threefold. A more precise way of expressing this effect is by means of the Arrhenius equation:

$$\frac{d \ln k}{dT} = \frac{E}{RT^2} \quad (7)$$

in which k is the reaction velocity at the absolute temperature, T , R is the gas constant, equal to 1.986 calories, and E is the energy of activation. The integral form of the equation is

$$\ln k = \frac{EC}{R} - \frac{E}{R} \left(\frac{1}{T} \right) \quad (8)$$

When, in accordance with this equation, values of $\ln k$ are plotted as ordinates against values of $\frac{1}{T}$ as abscissae, a straight line relationship is obtained, where $-E/R$ is the slope and EC/R is the intercept on the y-axis. This permits the calculation of E , the energy of activation. Many of the older texts and even some current texts and monographs list values for the energy of activation that are based on the use of incorrect measures of reaction velocity [19,37]. Most hydrolytic enzymes have correct values for E near 10,000 cal. per mole. The energy of activa-

tion may be considered as the amount of energy that molecules must absorb so that they can become "activated," become reactive, and be converted to their products. It is characteristic of enzyme-catalyzed reactions that their energy of activation is less than that for the corresponding non-enzymic reaction. For example, the energy of activation of sucrose inversion by hydrogen ions is 26,000 cal. per mole whereas that by yeast invertase is only 8,700 cal. per mole [26].

Inhibition. Inhibition of enzyme activity may either be reversible or irreversible. In the latter case, inhibition is usually progressive and the equilibrium is slowly attained. Perhaps the best known example of this type of inhibition is that of cholinesterase by diisopropylfluorophosphate. In reversible inhibition, as for example, that of phosphatase by amino acids [32], equilibrium is rapidly attained and the inhibition may be easily reversed, usually by dialysis. Reversible inhibitions may be competitive, non-competitive, uncompetitive, or combinations of these.

Fully competitive inhibition: In this type of inhibition (Figs. 2A and 2B) the substrate competes with inhibitor for enzyme so that the inhibition decreases with increasing substrate, and no inhibitory effect is present at infinite substrate concentration ($1/s = 0$). The general equation for this type of inhibition, expressed in terms of reciprocal velocities, is:

$$\frac{1}{v} = \frac{1}{V} \left[1 + \frac{K_s}{s} \left(1 + \frac{I}{K_I} \right) \right] \quad (9)$$

At varying substrate concentrations, the form would be

$$\frac{1}{v} = \frac{1}{V_{\max}} \left(K_s + \frac{K_s I}{K_I} \right) \frac{1}{s} + \frac{1}{V_{\max}} \quad (10)$$

I is the concentration of inhibitor; v is the velocity at substrate concentration, s ; and V is the maximal velocity when I is kept constant and s is varied, or is the velocity in the absence of inhibitor when I is varied at constant substrate concentration. Comparison with equation 3 shows that the dissociation constant of the enzyme-substrate complex in the presence of inhibitor is $K_p = K_s + K_s I/K_I$ (Fig. 2A.) K_I , the dissociation constant of the enzyme-inhibitor complex, is the projection of the x-axis of the point of crossing of plots of $1/v$ against I at different concentrations of substrates. (Fig. 2B.)

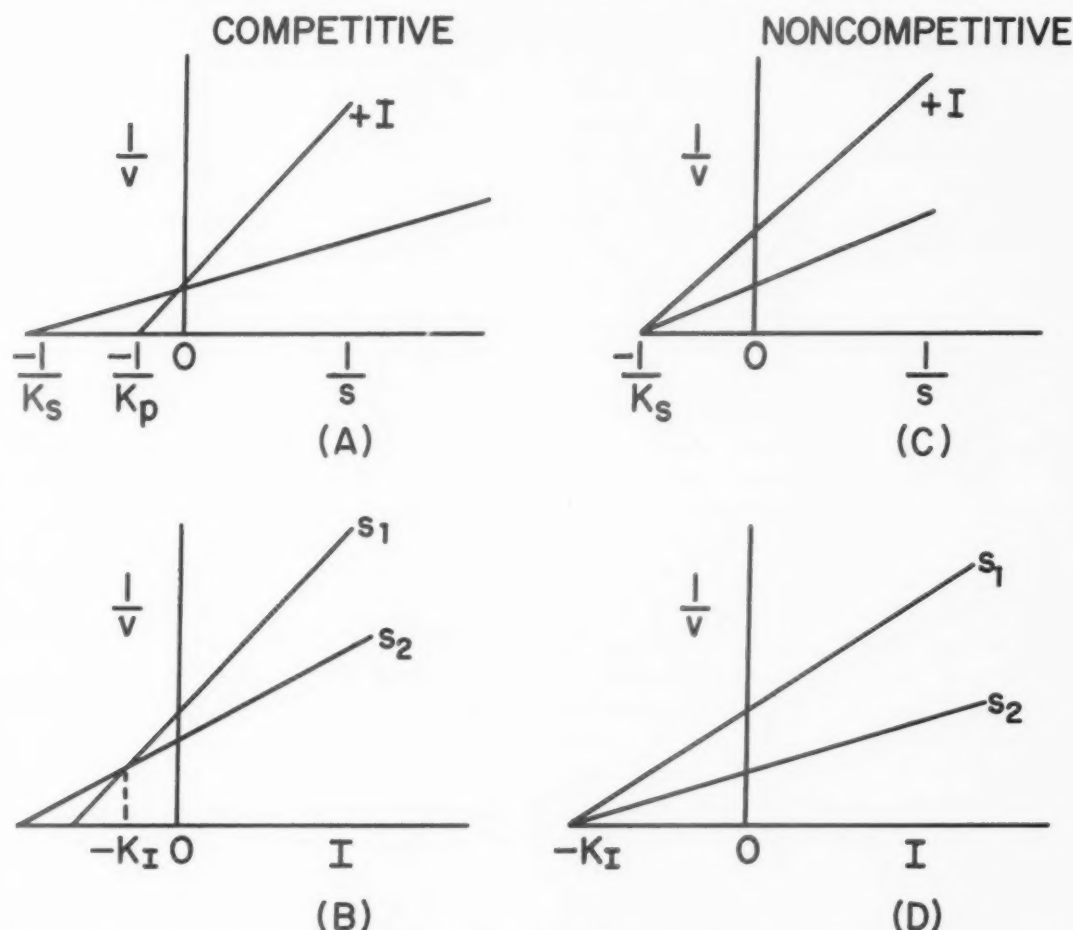


FIG. 2. Types of inhibition. A, plot of reciprocal of reaction velocity against reciprocal of substrate concentration to yield values of K_s and K_p . B, plot of reciprocal of reaction velocity against concentration of inhibitor to yield value of K_I . C, plot of reciprocal of reaction velocity against reciprocal of substrate concentration in case of noncompetitive inhibition to yield value of K_s . D, plot of reciprocal of reaction velocity against concentration of noncompetitive inhibitor to yield value of K_I .

Non-competitive inhibition: As the name indicates, the inhibitor does not compete with the substrate for enzyme, and at a constant concentration of inhibitor, the extent of inhibition is the same at all concentrations of substrate. The general expression for this type of inhibition, expressed in reciprocal form, is

$$\frac{1}{v} = \frac{1}{V} \left(1 + \frac{K_s}{s} \right) \left(1 + \frac{I}{K_I} \right) \quad (11)$$

where the terms have the significance described in the preceding paragraph. As is implicit in equation 11, and shown in Figures 2C and 2D, K_s has the same value at varying concentrations of inhibitor and $-K_I$ is the intercept on the x-axis when $1/v$ is plotted against I . An example of non-competitive inhibition is the effect of glycine on rat bone phosphatase, where K_I has a value of 0.039 mole per L. [32].

Other types of inhibition: Mixed and uncompetitive types of inhibition have also been reported. In the former, the straight line plot of $1/v$ against $1/s$ in the presence of inhibitor does not intersect the corresponding line in the absence of inhibitor either at $1/s = 0$, as in competitive inhibition (Fig. 2A) or at $1/s = -1/K_s$, as in non-competitive inhibition (Fig. 2C), but at some intermediate point. The inhibition of rat intestinal phosphatase by glycine affords an example of mixed inhibition [32]. In uncompetitive inhibition, the plot of $1/v$ against $1/s$ yields a straight line which is parallel to that obtained without inhibitor. This type of inhibition has been interpreted as indicating combination of the inhibitor only with the intermediate complex [33], and Dodgson and his associates [34] have shown that the enzyme-substrate complex may contain a dissociating group, not present in the enzyme, that may combine with the inhibitor.

BIOLOGICAL ASPECTS OF ENZYME ACTIVITY

Formation of Enzymes. The formation of enzymes in the organism has commanded wide interest in recent years. Only the mere outlines of this subject can be indicated in this review. Enzymes, like other proteins, are synthesized and degraded in the body. Isolation of aldolase, phosphorylase and glyceraldehyde 3-phosphate dehydrogenase from rabbit muscle after injection of labelled amino acids indicated that the turnover of these enzymes proceeded at the rate of .5 to 1 per cent of the total of each enzyme per day [35,36]. Synthesis of enzymes has also been shown to occur in tissue slices *in vitro* and in disrupted bacterial cells. For example, aerobic incubation of pigeon pancreas slices leads to the formation of lipase and ribonuclease [37].

The biosynthesis or induction of enzymes in bacterial cells has been found to be dependent upon the following factors: (1) the presence of an effective inducer, for example, a substrate or a structurally related substance; (2) the existence in the genetic mechanism of the cell of the appropriate gene for the formation of the particular enzyme; (3) the availability of the raw material, such as preformed protein or the various amino acids, from which the enzyme is formed; (4) the presence of a source of energy, such as a phosphorylation mechanism, for the synthesis of peptide bonds [1,38].

Of present relevance to medicine is the mechanism of formation of active enzymes from inactive protein precursors, such as pepsin from pepsinogen, trypsin from trypsinogen, rennin from prorennin, carboxypeptidase from procarboxypeptidase and chymotrypsin from trypsinogen. The process of blood clotting and lysis also involves sets of enzymes and inactive precursors. In general, it would appear that the transformation from precursor to active enzyme consists in the breaking of peptide links, with or without the removal of free peptides.

Cell Structure. Considerable work of a quantitative and semiquantitative nature has been carried out on the distribution of enzymes among the various intracellular structures—the nucleus, mitochondria, microsomes—and the supernatant fraction remaining after the centrifugation of these particulate structures [39,40]. For example, in the rodent liver the nucleus contains few enzymes, and most of these, like arginase or 5-nucleotidase, to only a small extent. The fol-

lowing enzymes are present chiefly in the mitochondria: acid phosphatase, desoxyribonuclease II, L-glutamate dehydrogenase, isocitric dehydrogenase (Co I-specific), succinic dehydrogenase, cytochrome oxidase, adenylic kinase, acetyl-Co A transacylase; adenosinetriphosphatase is also present in substantial amounts in this fraction. Among the enzymes present mainly in the microsomes are: alkaline phosphatase, cholinesterase, cholesterol esterase and glucose-6-phosphatase. The supernatant fraction contains chiefly enzymes of the glycolytic cycle, like phosphoglucomutase, aldolase and lactic dehydrogenase, but also enzymes of other metabolic sequences such as glutamic oxaloacetic transaminase, adenosine deaminase and aconitase.

The localization of certain groups of enzymes indicates the association of metabolic function with intracellular structure. For example, mitochondria contain the requisite groups of enzymes catalyzing the tricarboxylic acid cycle, the oxidation of fatty acids, the transfer of electrons from substrate to oxygen, and oxidative phosphorylation [41]. The enzymes of the glycolytic cycle are present chiefly in the supernatant fraction. However, several of the enzymes involved in these metabolic sequences are not confined to one fraction, and further work remains to be done on relating metabolic function to structure.

Enzymology and Genetics. The idea that genes are involved in the formation of enzymes occurred several times in the formulations of geneticists and workers from other disciplines in the early part of this century [42]. These early conceptions, formulated as the "one gene-one enzyme" hypothesis, have been developed brilliantly during the past fifteen years by studies on neurospora and other molds. In man, genetic defects may be reflected in the loss or decrease of an enzyme in essential metabolic sequences. Of interest is the finding that these inborn errors of metabolism are manifested by the absence of a specific enzyme from the tissues, serum or erythrocytes, or by the piling up of metabolites in the blood. As Gutman has pointed out, the clinical expression of these defects is "the result of a complex interaction of genetic, metabolic and environmental influences involving the interplay of diet, endocrine functions, intercurrent diseases, the action of drugs, emotional stresses and other factors" [43].

Some of the hereditary diseases in which enzyme defects have been demonstrated, and the

tissues in which these defects have been found, are [44]: albinism, probably tyrosinase in skin; alkaptonuria, homogentisate oxidase in liver and perhaps kidney; congenital hyperbilirubinemia, bilirubin-glucuronyl transferase in liver and kidney; galactosemia, galactose-1-phosphate uridylyl transferase in red blood cells and liver; glycogen storage disease (type A), glucose-6-phosphatase in liver and kidney; glycogen storage disease (type B), brancher enzyme in liver; glycogen storage disease (type C), debrancher enzyme in liver; hypophosphatasia, alkaline phosphatase in serum, white blood cells, bone, intestine, kidney and liver; phenylpyruvic oligophrenia, phenylalanine hydroxylase in liver; hemolytic anemias in drug-sensitive persons, glucose-6-phosphate dehydrogenase in erythrocytes. These demonstrations of enzymic defects in clearly hereditary diseases suggest the possibility that other diseases in which hereditary factors are often implicated may also be characterized, albeit to a lesser degree, by partial enzyme defects.

General Relation of Tissue to Serum Enzymes. Many enzymes have been found in the serum. Calculations from the probable molecular weight and enzyme activity of aldolase and of desoxyribonuclease have indicated that about 5 mg. of an enzyme protein are normally present in the circulation of man [45]. Some enzymes in the serum, such as alkaline phosphatase or transaminase, are mixtures of enzymes derived from several tissues. The precise mechanisms by which the enzyme protein passes from the cell into the extracellular fluid are not known precisely, but some of the over-all factors appear to be secretion by a specific tissue, damage or destruction of tissue with resulting leakage of enzyme protein, and removal from the circulation by excretion or degradation. Obviously, one or more of these mechanisms may be altered greatly in disease.

REFERENCES

1. DIXON, M. and WEBB, E. C. *Enzymes*. New York, 1958. Academic Press, Inc.
2. RYLE, A. P., SANGER, F., SMITH, L. F. and KITAI, R. The disulphide bonds of insulin. *Biochem. J.*, 60: 541, 1955.
3. SANGER, F. The arrangement of amino acids in proteins. *Advances Protein Chem.*, 7: 1, 1952.
4. HIRS, C. H. W., STEIN, W. H. and MOORE, S. Studies obtained by chymotryptic hydrolysis of performic acid-oxidized ribonuclease. A partial structural formula for the oxidized protein. *J. Biol. Chem.*, 221: 151, 1956.
5. REDFIELD, R. R. and ANFINSEN, C. B. The structure of ribonuclease. II. The preparation, separation, and relative alignment of large enzymatically produced fragments. *J. Biol. Chem.*, 221: 385, 1956.
6. ANFINSEN, C. B. The limited digestion of ribonuclease with pepsin. *J. Biol. Chem.*, 221: 405, 1956.
7. HILL, R. L. and SMITH, E. L. Crystalline papain. VI. Extensive stepwise hydrolysis of mercuripapain by leucine aminopeptidase without loss of proteolytic activity. *Biochim. et biophys. acta*, 19: 376, 1956.
8. KENNEDY, E. P. and KOSHLAND, D. E., JR. Properties of the phosphorylated active site of phosphoglucomutase. *J. Biol. Chem.*, 228: 419, 1957.
9. WHITE, A., HANDLER, P., SMITH, E. L. and STETTEN, DE WITT, JR. *Principles of Biochemistry*. New York, 1954. McGraw-Hill Book Co., Inc.
10. LEHNINGER, A. L. Roles of metal ions in enzyme systems. *Physiol. Rev.*, 30: 393, 1950.
11. RABIN, B. R. and CROOK, E. M. The activation of enzymes by metal ions. *Biochim. et biophys. acta*, 19: 550, 1956.
12. MALMSTRÖM, B. G. The interaction of purified enolase with its activating metal ions. *Arch. Biochem.*, 46: 345, 1953.
13. SMITH, R. M. and ALBERTY, R. A. The apparent stability constants of ionic complexes of various adenosine phosphatases with different cations. *J. Am. Chem. Soc.*, 78: 2376, 1956.
14. BODANSKY, O. The accelerant effect of α -amino acids on the activity of bone phosphatase. *J. Biol. Chem.*, 114: 273, 1936.
15. SUTHERLAND, E. W. Activation of phosphoglucomutase by metal-binding agents. *J. Biol. Chem.*, 180: 1279, 1949.
16. SIDBURY, J. B. and NAJJAR, V. A. Further studies on the mechanism of phosphoglucomutase: the phosphoenzyme bond. *J. Biol. Chem.*, 237: 517, 1957.
17. BODANSKY, O. The use of different measures of reaction velocity in the study of the kinetics of biochemical reactions. *J. Biol. Chem.*, 120: 555, 1937.
18. BODANSKY, O. Measurement of enzyme activity at any stage of a reaction. *J. Biol. Chem.*, 205: 731, 1953.
19. SUMNER, J. B. and SOMERS, G. F. *Chemistry and Methods of Enzymes*, 3rd ed. New York, 1953. Academic Press.
20. MOELWYN-HUGHES, E. A. *The Kinetics of Reactions in Solution*, 2nd ed. Oxford, 1947. Clarendon Press.
21. SCHÜTZ, E. Eine Methode zur Bestimmung der relativen Pepsinmenge. *Ztschr. physiol. Chem.*, 9: 577, 1885.
22. BODANSKY, O. The Schütz-Borrisov law for enzymes. *Science*, 86: 52, 1937.
23. STRAUB, F. B. On the kinetics of lactic dehydrogenase. *Enzymologia*, 9: 143, 1940.
24. MICHAELIS, L. and MENTEN, M. L. Die Kinetik der Invertinwirkung. *Biochem. Ztschr.*, 49: 333, 1913.
25. LINEWEAVER, H. and BURK, D. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.*, 56: 658, 1934.
26. NELSON, J. M. and BLOOMFIELD, G. Some characteristics of invertase action. *J. Am. Chem. Soc.*, 46: 1025, 1924.

27. LAIDLER, K. J. The influence of pH on the rate of enzyme reactions. Part 1—General theory. Part 2—The nature of the enzyme-substrate interaction. *Tr. Faraday Soc.*, 51: 528, 540, 1955.
28. HASE, E. Studies on the mechanism of action of cholinesterase. I. Kinetic studies on the action of hydrogen ion upon cholinesterase. *J. Biochem.*, 39: 259, 1952.
29. BULL, H. B. and CURRIE, B. T. Peptic hydrolysis of egg albumin. I. Kinetic studies. *J. Am. Chem. Soc.*, 71: 2758, 1949.
30. SIZER, I. W. Effects of temperature on enzyme kinetics. *Advances in Enzymology*, 3: 35, 1943.
31. BODANSKY, O. The energy of activation of the hydrolysis of sodium β -glycerophosphate by bone phosphatase at optimal pH. *J. Biol. Chem.*, 129: 197, 1939.
32. BODANSKY, O. Mechanism of inhibition of phosphatase activity by glycine. *J. Biol. Chem.*, 165: 605, 1946.
33. EBERSOLE, E. R., GUTTENTAG, G. and WILSON, P. W. Nature of carbon monoxide inhibition of biological nitrogen fixation. *Arch. Biochem.*, 3: 399, 1943.
34. DODGSON, K. S., SPENCER, B. and WILLIAMS, K. Examples of anticompetitive inhibition. *Nature, London*, 177: 432, 1956.
35. SIMPSON, M. V. and VELICK, S. F. The synthesis of aldolase and glyceraldehyde-3-phosphate dehydrogenase in the rabbit. *J. Biol. Chem.*, 208: 61, 1954.
36. HEIMBERG, M. and VELICK, S. F. The synthesis of aldolase and phosphorylase in rabbits. *J. Biol. Chem.*, 208: 725, 1954.
37. SCHUCHER, R. and HOKIN, L. E. The synthesis and secretion of lipase and ribonuclease by pigeon pancreas slices. *J. Biol. Chem.*, 210: 551, 1954.
38. MONOD, J. Remarks on the mechanism of enzyme induction. In: *Enzymes: Units of Biological Structure and Function*. Edited by Gaebler, O. H. New York, 1956. Academic Press.
39. DE DUVE, C., PRESSMAN, B. C., GIANETTO, R., WATTIAUX, R. and APPELMANS, F. Tissue fractionation studies. VI. Intracellular distribution pattern of enzymes in rat liver tissue. *Biochem. J.*, 60: 604, 1955.
40. DOUNCE, A. L. Cytochemical foundations of enzyme chemistry. In: *The Enzymes*, vol. 1. Edited by Sumner, J. B. and Myrback, K. New York, 1950. Academic Press.
41. LEHNINGER, A. L. Physiology of mitochondria. In: *Enzymes: Units of Biological Structure and Function*. Edited by Gaebler, O. H. New York, 1956. Academic Press.
42. TATUM, E. L. The status of the gene-enzyme relationship. In: *Enzymes: Units of Biological Structure and Function*. Edited by Gaebler, O. H. New York, 1956. Academic Press.
43. GUTMAN, A. B. The expanding pattern of the inborn errors of metabolism. *Bull. New York Acad. Med.*, 35: 419, 1959.
44. KRETCHMER, N., STONE, M. and BAUER, C. Hereditary enzymatic effects as illustrated by hypophosphatasia. *Ann. New York Acad. Sc.*, 75: 279, 1958.
45. BODANSKY, O. Enzymes and cancer (panel). Public Health Service Publication, No. 96, 1951.

Serum Alkaline Phosphatase Activity in Diseases of the Skeletal and Hepatobiliary Systems*

A Consideration of the Current Status

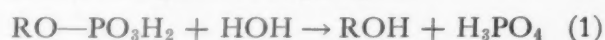
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THE blood plasma of normal man contains representatives of two classes of "non-specific" phosphomonoesterases, one exhibiting optimum activity at pH ca. 9 with substrate in high concentration [1,2], the other at pH ca. 5 [3]—hence their designation, conveniently if inexactly, as alkaline and acid phosphatases. The proteins endowed with these enzymic properties are separated with the alpha-globulins by alcohol fractionation of the plasma proteins [4]. Paper electrophoresis of the serum of normal human subjects shows maximal phosphatase activity migrating with the alpha-2-globulin peak [5-8]; in some hands, however, appreciable activity was found to separate also with the beta-globulins [5]. Starch gel electrophoresis of normal human serum likewise indicates a peak of serum alkaline phosphatase activity in the region of the alpha-2-globulins, in most instances associated with a diffuse trail in the beta-globulin zone and slight activity, averaging 1.8 per cent of the total, in the alpha-1-globulin region [9]. Similar findings [10,11] have been interpreted, however, as two distinct peaks of alkaline phosphatase activity in normal human serum, the major peak in the beta-globulin area, a lesser peak with the alpha-2-globulins. Clarification of these discrepancies in interpretation must await further study.

The "non-specific" phosphatases, unlike the "specific" phosphatases (such as glucose-6-phosphatase) which are more selective in their substrates, act upon the orthophosphoric monoesters of a wide variety of phenolic, alcoholic, sugar and other compounds [12]. Two types of reactions are catalyzed. In one the enzyme acts

as a hydrolase, with cleavage of the P—O bond [13] and removal of the phosphoryl group to liberate inorganic orthophosphate:



The second type of reaction, a transphosphorylation in which the enzyme acts as a phosphotransferase, does not involve the intermediate formation of inorganic phosphate in transmission of the phosphoryl group to the acceptor [13]:



This reaction, which results in phosphoric ester synthesis [14], does not require the presence of adenosine triphosphate or other high-energy phosphate compounds [15]. It should be noted that reaction (1) is really a special case of reaction (2).

It has been generally assumed that reaction (1) characterizes the action of alkaline phosphatase in its natural environment, and indeed all methods of estimating the enzyme are based on this premise, but the assumption may not prove to be altogether valid. Whether the enzyme predominantly catalyzes reactions (1) or (2) appears to depend upon competition between water and other hydroxyl-containing compounds for sites at the surface of the enzyme-donor complex [15]; this is determined chiefly by the relative concentrations of the participating compounds and the velocity constants of the two reactions at the pH of the environment. These factors are important in considerations of the still obscure role of alkaline phosphatase in

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nature. At very low concentrations of substrate, such as may be presumed to obtain intracellularly, it has been shown that, at least for intestinal mucosa alkaline phosphatase [16], the optimum pH falls to 7.35. Histochemical studies [17] of the liver under similar "physiological" conditions of substrate concentration and pH reveal more alkaline phosphatase activity of the hepatic parenchymal cell cytoplasm than by the standard Gomori method—if specificity of enzyme action under these physiological conditions is conceded.

Both reactions (1) and (2) clearly involve the formation of an intermediate enzyme-phospho compound. Nothing definite is known as yet, however, of the substrate-binding groups of the active center of the enzyme, nor has the significance of the action of metal ion activators, such as Mg^{++} [18], or of other activators and inhibitors been fully elucidated. These and other aspects of the nature and action of the phosphatases are discussed in a number of reviews [13,15,19-22].

EARLY STUDIES FOCUSING INTEREST ON THE SKELETAL AND HEPATOBILIARY SYSTEMS

Robison's discovery of alkaline phosphatase in bone [23], and his theory of the role of this enzyme in bone formation [24,25], stimulated investigation of the effect of skeletal diseases on the serum alkaline phosphatase. Rewarding results were soon forthcoming. Markedly increased alkaline phosphatase activity of the serum was demonstrated in Paget's disease [26,27], osteitis fibrosa (hyperparathyroidism) [26], rickets [27,28], osteomalacia [28], osteosarcoma [26] overproductive of bone [29], and carcinoma with widespread skeletal metastases [26] which are predominantly osteoplastic [30]. It was first thought [26] that the increase in serum alkaline phosphatase activity in these and not in most other skeletal disorders was a reflection simply of their wide skeletal involvement but it was soon discovered that the productive nature as well as the dissemination of the process was of importance in this connection. Examination of involved tissues in the disorders in question revealed increased alkaline phosphatase activity of the calcifying cartilage in rickets [37], and of bone at the site of the affection in Paget's disease [29,32], osteosarcoma overproductive of bone [29] and osteoplastic metastases secondary to prostatic carcinoma [32]. It was thus made apparent that enhanced osteoblastic

(chondrocytic) activity at the involved skeletal sites, and the resulting local overproduction of bone alkaline phosphatase in these disorders (if sufficiently in excess), was responsible for the surplus alkaline phosphatase appearing in the plasma. Correlative studies on the association of the enzyme with bone formation in ontogenesis and phylogenesis, the results of *in vitro* tissue culture, and a variety of other lines of investigation gave further support to the concept relating bone (cartilage) alkaline phosphatase to normal and abnormal bone formation, and to the increased serum alkaline phosphatase in certain skeletal disorders. These points and many others are more fully elaborated in reviews of the period [19,24,30,33-40].

A second category of diseases, involving the hepatobiliary system, was found to be associated with increased phosphatase activity of the plasma by Roberts [27,47], who noted augmented values in patients with jaundice due to obstruction of the extrahepatic biliary tract and normal or slightly increased levels in patients with catarrhal, infective, toxic and hemolytic jaundice. Roberts [47] proposed use of the determination to distinguish obstructive from other forms of jaundice. The increased serum alkaline phosphatase in patients with obstructive jaundice was fully substantiated [42-51]; and, indeed, soon reproduced in the dog by obstructing the common bile duct [42,52], with rapid return to normal levels when the obstruction was relieved, in both dog and man. However, increased (and overlapping) serum alkaline phosphatase values were recorded also in cases of hepatogenous jaundice by A. Bodansky and Jaffe [53], and by many others [43,45,46,48,49, 51,54]; in the opinion of some [43,49,54, and others] this overlap was of such proportions as to disqualify use of the determination to differentiate obstructive from hepatogenous jaundice. On the other hand, the level of serum alkaline phosphatase activity was found to be a more useful index of certain forms of hepatobiliary disease than was at first appreciated, since it reflects obstruction not only of the extrahepatic biliary tract, with or without overt jaundice, but also obstruction of the intrahepatic biliary tract, with or without jaundice. This last was first noted in connection with carcinoma metastatic to the liver [30,48,55], subsequently also with a variety of granulomatous and infiltrative diseases of the liver in advance of significant hyperbilirubinemia [51,56], and following ex-

TABLE I
RANGE OF NORMAL VALUES FOR SERUM ALKALINE PHOSPHATASE ACTIVITY IN ADULT MAN, BY
METHODS CURRENTLY IN GENERAL USE

| Method | Substrate | Unit | Normal Range |
|-------------------------------|--------------------------------|------------------------------------|----------------------------|
| Bodansky [65] | β -Glycerophosphate | 1 mg. P/100 ml./60' | 1.5-4.0 |
| King-Armstrong [66] | Phenylphosphate | 1 mg. phenol/100 ml./30' | 3 -13 |
| Shinowara-Jones-Reinhart [67] | β -Glycerophosphate | 1 mg. P/100 ml./60' | 2.8-8.6 |
| Bessey-Lowry-Brock [68] | <i>p</i> -Nitrophenylphosphate | 1 mM <i>p</i> -nitrophenol/100/30' | 1.8 \times Bodansky unit |

posure to drugs causing a hypersensitivity reaction in the liver characterized by marked intrahepatic cholestasis [57]. It was concluded from these early studies that the determination of serum alkaline phosphatase affords, in man, a sensitive criterion of the patency of the excretory biliary channels, extrahepatic and intrahepatic [57].

By 1940, investigation of the serum alkaline phosphatase had thus revealed markedly increased levels of activity in two general categories of human disease: disorders of the skeleton characterized by overactivity of substantial numbers of osteoblasts (or chondrocytes), and disorders of the hepatobiliary system, notably those characterized by obstruction of the extrahepatic or intrahepatic biliary tract. Broad surveys of all the ills of mankind before 1940, and since, have failed to disclose any other diseases regularly associated with striking increases of the enzyme in the plasma, except as might be conceived to result from an impact upon one or both of the two systems mentioned. The remainder of this discussion will therefore be restricted to a closer examination of serum alkaline phosphatase levels in the relevant diseases of the skeletal and hepatobiliary systems; a consideration of the mechanisms whereby the circulating enzyme is increased in diseases of these two systems, and no other; and a brief summary of the present inchoate state of knowledge concerning the physiological role of the enzyme. Aspects of these topics are further discussed in the reviews already cited, and elsewhere [58-62].

SERUM ALKALINE PHOSPHATASE ACTIVITY IN SKELETAL DISORDERS

Paget's Disease. Increased alkaline phosphatase activity is the most pronounced and consistent abnormality of the blood plasma in Paget's disease [30], and in a related disorder,

osteoporosis circumscripta of the skull [63,64]. The enzyme activity varies widely in different cases, from within or moderately above the normal range (Table I) to values as high as are encountered in any disease, i.e., in excess of 100 Bodansky units or 200 King-Armstrong units; depending for the most part upon the extent of skeletal involvement [28,30,34,37,69-75], and the activity of the lesions [30,34,69,71,72]. In a representative series of seventy-six patients with Paget's disease [30] the serum alkaline phosphatase activity in the most advanced and widespread cases ranged from 60 to 130 (mean 106) Bodansky units; in those with somewhat less extensive involvement, from 10 to 120 (mean 49) Bodansky units; and in those with more localized disease, from 2 to 50 (mean 12) Bodansky units. Once a steady state of serum enzyme activity is established in the affected individual, there is usually comparatively little change over a period of a year or two [72,75,76], but with more protracted observation there is apt to be a slow rise with extension of the disease [75] or a more rapid increase when there is development of osteogenic sarcoma [72,75]. Administration of ACTH or cortisone in high dosage causes a transitory fall in serum alkaline phosphatase activity [77], often followed by a sharp rebound.

Although increased serum alkaline phosphatase activity is the sole known characteristic of the blood in Paget's disease, and may be very marked, its lack of specificity, at least as an isolated diagnostic test, limits the value of the determination in differential diagnosis. It may be found useful in the detection of unsuspected Paget's disease and in confirmation of the diagnosis which, however, can be made earlier and with more assurance by roentgenographic examination. The sometimes difficult roentgenographic differentiation of Paget's disease from the osteoplastic metastases of prostatic

carcinoma is not aided by the determination of serum alkaline phosphatase, since high values are common to both. Here the serum acid phosphatase is useful in diagnosis, although extensive Paget's disease with high serum alkaline phosphatase may also be associated with a minor increase in serum acid phosphatase activity [78]; not because of residual alkaline phosphatase activity (which is completely abolished at pH 5.0 [3]), but probably due to mobilization of bone acid phosphatase [78], which appears to be related to enhanced osteoclastic activity [78-81]. In the prognosis of Paget's disease, the determination of serum alkaline phosphatase may indicate relative quiescence or activity of the process, if the values are unduly low or high in respect to the extent of disease. Sharply rising levels may portend sarcomatous transformation.

Hyperparathyroidism. The serum alkaline phosphatase activity in this disorder varies from within normal limits to values approaching 100 Bodansky units or 200 King-Armstrong units, but the mean figures are substantially lower than in Paget's disease. Early case reports, recorded when the diagnosis was dependent upon the presence of extensive skeletal lesions, stressed the consistency of associated increases in serum alkaline phosphatase activity and the usefulness of the determination in differentiation from other diseases accompanied by hypercalcemia, such as multiple myeloma and carcinoma with extensive osteolytic metastases [30]. This still holds, but before the skeleton is overtly affected the values are normal or only equivocally increased [82,83]; hence the determination is of no aid in early diagnosis. This dependence of augmented serum alkaline phosphatase activity upon the presence of skeletal involvement in hyperparathyroidism accords with the view that the enzyme originates from osteoblasts but the predominately lytic nature of generalized osteitis fibrosa cystica might seem to be inconsistent. Microscopic examination of the bone, however, reveals enhanced, if largely abortive, new bone formation which roughly corresponds in degree to the height of the serum alkaline phosphatase [82,83]. The skeletal origin of the surplus serum enzyme is indicated also by the fact that upon removal of the offending parathyroid tumor the increase in serum alkaline phosphatase persists, and may even rise further temporarily [30], subsiding gradually over a period of many months as the processes of bone repair slowly come to a halt. This is in sharp

contrast to the abrupt cessation of osteolysis and the rapid restoration of normal levels of serum calcium, phosphorus and acid phosphatase (this last may be somewhat elevated [78,84], for reasons already indicated). Similar conclusions may be drawn from the increased serum alkaline phosphatase activity occurring in hyperparathyroidism experimentally produced in dogs and guinea pigs by repeated injections of parathyroid extract [85,86].

Rickets and Osteomalacia. A distinct rise in serum alkaline phosphatase activity accompanies florid rickets in the child and osteomalacia in the adult, whether due to vitamin D deficiency or to defective reabsorption in the renal tubules or gut. In malnutritional rickets [87-94] this increase in serum enzyme is usually the first index of active disease, more sensitive than clinical evaluation, roentgenographic examination, even the serum inorganic phosphate level [88,89,93,94]. The degree of augmentation of serum alkaline phosphatase activity generally reflects the severity of rickets; many exceptions occur but are as likely as not to be due to the shortcomings of clinical evaluation of rachitic activity [89,92,94]. Values up to about 20 Bodansky units (30 to 40 King-Armstrong units) characterize the initial stages of the disorder [89,93], and a progressive rise in serum enzyme activity (to 20 to 60 Bodansky units [89]) parallels advance of the disease, to reach levels approximating 200 units in the most florid stages [89,94]. Administration of vitamin D, if adequate, is followed by declining levels of serum alkaline phosphatase activity as healing occurs [89-94]; but in the more severe cases there is a temporary secondary rise after the initial fall, finally a slow return to normal values [94]. So faithfully does the enzyme response mirror dosage that it has been utilized in the rachitic chick to assay vitamin D preparations [95,96]. In this species also it has been possible to demonstrate that as the vitamin D content of the diet is diminished there is a parallel rise in the alkaline phosphatase of the bones and plasma whereas the alkaline phosphatase activity of the liver, kidney and intestinal mucosa remain unaffected [97]; this and other [98] evidence is in accord with the skeletal origin of the increase in plasma alkaline phosphatase characterizing rickets due to hypovitaminosis D.

In late rickets and osteomalacia the skeletal derangement is apt to be less extensive, and the rise in serum alkaline phosphatase correspond-

ingly less marked. In the endemic form of the disorder [67] a range of 8 to 15 Bodansky units has been recorded [99], with values up to about 30 Bodansky units in more severe cases [100]. In osteomalacia (Milkman's syndrome) encountered in the Occident, usually related to malabsorption syndromes or renal tubular defects, the serum alkaline phosphatase activity is increased as a rule but only occasionally exceeds 20 Bodansky units or 30 King-Armstrong units [101-104]. Values as high as 45 Bodansky units and 60 King-Armstrong units occur in children with vitamin D-resistant rickets secondary to the Fanconi syndrome [105,106].

Osteosarcoma. The serum alkaline phosphatase activity in bone sarcoma varies from within or only moderately above the normal range, particularly in predominantly lytic tumors [30,36], to very substantial levels in osteogenic types with widespread osteoplastic metastases. There is a general but not altogether consistent correlation between the serum enzyme level and the tumor enzyme concentration [80], which may be very high [29,80]—of the order of 200 units of alkaline phosphatase activity per gram [80] as compared with some 0.5 units per gram of normal bone. Tissue extraction [29,80] and histochemical studies [107] leave no doubt that the surplus enzyme in the serum originates in the osteoblasts of the neoplasm. Moreover, when the primary tumor is removed by amputation the serum alkaline phosphatase falls rather abruptly [29,36,40], only to soar again with the appearance of fresh metastases, which usually are about as rich in the enzyme as their primary congener [80].

Carcinoma Metastatic to Bone. Subsequent experience [108] has sustained the principle [30,37] that predominantly osteoplastic metastases to bone are associated with more consistent and marked elevations in serum alkaline phosphatase activity than are lytic skeletal lesions, although increases in the latter group may accompany hepatic metastases and new bone formation not apparent roentgenographically. In any event, the prime tumor responsible for the highest serum enzyme levels is prostatic carcinoma when there is extensive osteoplastic involvement of the skeleton. Woodard [108] gives a mean value of 20.7 Bodansky units for 110 cases of prostatic carcinoma with metastases to the bone (as compared to 6.8 units for 443 cases of mammary carcinoma with skeletal spread and 8.5 units for 144 miscellaneous

tumors with secondary involvement of bone), and values as high as 120 [30] and 147 Bodansky units [108] have been recorded. In a survey of 560 patients with metastasizing prostatic carcinoma [109], 85.8 per cent had increased serum alkaline phosphatase activity, and in another large experience [108,110,111] the figure approximated 90 per cent in untreated patients. (This is a higher incidence than has been generally reported for increased serum acid phosphatase activity in patients with metastasizing prostatic carcinoma [from 65.5 per cent [109] to the more usual 80 per cent [59]]; however, the rise in serum acid phosphatase has, of course, more specific diagnostic implications.)

There can be little question as to the osteoblastic origin of the excess serum alkaline phosphatase activity under these circumstances. This is indicated by the association of markedly increased serum enzyme levels with predominantly osteoplastic metastases, rather than with osteolytic metastases or non-metastasizing tumor; the enhanced enzyme content of the osseous tissue at the site of such osteoplastic metastases [32,80]; and the effect of therapy on the serum enzyme level. Whereas orchiectomy or steroid administration is promptly followed by a precipitous decline in serum acid phosphatase [112], the serum alkaline phosphatase activity is but little affected, indeed it often rises temporarily [78,110-113], to recede slowly over many months, usually to essentially normal levels as the processes of skeletal repair gradually are completed; recurrence in the majority of cases is heralded by a rise in both acid and alkaline phosphatases in the serum. Essentially the same pattern of response in serum alkaline phosphatase activity is seen in carcinoma of the breast metastasizing to bone when endocrine therapy is instituted [114].

Miscellaneous Skeletal Disorders. Healing fractures generate substantial increases in bone phosphatase at the site of osteoblastic bone repair [115-117] but there is little or no associated rise in serum alkaline phosphatase [118-120], presumably because of the limited extent of the involved skeletal areas. In osteopetrosis (Albers-Schönberg disease) the serum alkaline phosphatase is usually within normal limits or but slightly increased [34,78,121], despite sometimes quite extensive skeletal sclerosis; in this obscure disorder, moreover, the serum acid phosphatase may rise perceptibly [78,121,122]. Generalized osteosclerosis due to chronic

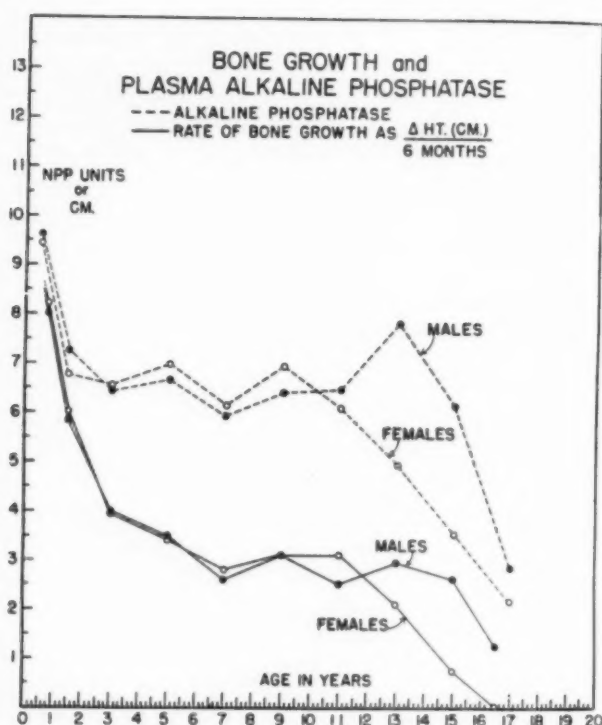


FIG. 1. Relationship of plasma alkaline phosphatase activity to skeletal growth in man. From: L. C. CLARK and E. BECK, *J. Pediatr.*, 36: 335, 1950 [128].

fluorine poisoning in man also is associated with normal serum alkaline phosphatase levels [123]. In other forms of osteosclerosis, due to myeloproliferative disorders, lymphoma, and the like, the serum alkaline phosphatase activity may be somewhat increased but is often quite normal. In fibrous dysplasia of bone the findings also are variable, normal serum alkaline phosphatase activity occurring in three-fourths of the cases in one series [80], and in one-third of another series [124]; however, in one patient with extensive involvement of the skull values of 22 to 28 Bodansky units were obtained [125].

Diseases characterized by osteoporosis or bone destruction, with feeble or no reparative reaction, are quite uniformly accompanied by essentially normal serum alkaline phosphatase activity, even though the skeleton be extensively affected. A prime example is multiple myeloma; in eighty-three cases the serum alkaline phosphatase activity ranged from 1.3 to 12.1 (mean, 3.8) Bodansky units [108].

Skeletal Disorders Associated with Less Than Normal Serum Alkaline Phosphatase. The normal processes of growth are accompanied by commensurate serum alkaline phosphatase levels [87-89,126-128], in such extraordinarily close correspondence (Fig. 1) as to leave no doubt of the skeletal origin of the excess serum enzyme.

Thus the rapid growth of infancy is associated with the highest values (mean, approximately 8 Bessey-Lowry-Brock (B-L-B) units), which fall progressively and by the second year of life reach a plateau at 6 to 7 B-L-B units that is maintained into pubescence, when it declines to adult levels of 2 to 3 B-L-B units [128]. Disturbance of the skeletal growth pattern, of whatever etiology, is reflected in the serum alkaline phosphatase levels; for example, arrest of growth due to achondroplasia [34,88], cretinism [34,88,129,130] or other cause is accompanied by a fall in serum alkaline phosphatase often to adult levels whereas abnormal growth in gigantism with failure of epiphyseal closure is characterized by persistence of high childhood levels [34]. Of interest in this connection is scurvy [88,137], in which the decline in serum alkaline phosphatase activity may be disproportionate to the retardation in growth, apparently because some specific defect in osteoblastic function may be interposed [131,132]. Administration of vitamin C rapidly restores the serum enzyme level to normal. Of interest also is the lowering of the serum alkaline phosphatase activity occurring after exposure to radioactive substances which are deposited in the bone [133], where osteoblasts presumably are first inactivated and bone necrosis may then ensue. But doubtless the most intriguing disorder distinguished by low serum alkaline phosphatase activity is hypophosphatasia [134-142], an inborn error of metabolism characterized by deficiency of alkaline phosphatase in the bone, kidney, intestinal mucosa, liver, leukocytes and blood plasma (this last being reduced, on the average, to about one-fourth of the lower limit of normal [137]). The clinical syndrome is dominated by abnormal mineralization of bone, which may result in profound skeletal changes mimicking vitamin D-resistant rickets; another indication of the relationship between osteoblastic activity and the level of serum alkaline phosphatase activity. An aspect of the metabolic error which has evoked particular comment is the appearance in the urine of substantial quantities of phosphorylethanolamine, which therefore has been suggested as a natural substrate of bone alkaline phosphatase [140].

SERUM ALKALINE PHOSPHATASE ACTIVITY IN DISORDERS OF THE HEPATOBILIARY SYSTEM

In the early days of development of "liver function" tests it was hoped that a single, all-

purpose test could be devised which would reliably differentiate the various disorders of the liver and biliary tract. Innumerable such tests have been described [143,144], but not one of them has qualified, of itself, for this universal purpose or, as is now appreciated, could possibly be expected to provide all the information requisite for so complex a differentiation. With this realization came a trend to the other extreme, to veritable "batteries" of tests, seeking safety in numbers by the use, it would seem, of every available test even remotely involving the differential diagnosis of hepatobiliary disease. Apart from distracting from the essential of considered clinical analysis on the basis of history and physical examination, this multiplicity of measurements obviously is wasteful; moreover, as soon was found [51], the possibility of error cannot be minimized by a plethora of tests reduplicating much the same limitations. A more discriminating approach therefore was developed, employing specific tests for specific purposes, as necessitated by the particular problem at hand.

Virtually all such diagnostic problems incorporate at least three basic requirements: determination of the serum bilirubin, some means of assessment of the general state of the liver parenchyma, and some complementary measure of the patency of the biliary tract [145]; to which are added appropriately selected tests as needed, such as the determination of serum proteins in cirrhosis of the liver. By such purposeful combination the number of liver function tests can be reduced to a minimum, and the accuracy of differential diagnosis enhanced by the mutual check inherent in the use of complementary tests for hepatogenous and obstructive jaundice [145-153]. For example, MacLagen [152], in a study of 200 cases of jaundice, found that joint application of the thymol flocculation test and the serum alkaline phosphatase determination gave useful results in about 80 per cent of cases; in the remainder, the findings were either equivocal or such as not to assist in differential diagnosis. This would seem to be a realistic appraisal of what can be accomplished in unselected cases of jaundice by appropriate use of liver function tests alone, although others have reported even better results, particularly by serial measurements.

For any such combination of tests several sensitive criteria of disturbed liver parenchyma are available, albeit individual preferences have been expressed for the cephalin flocculation test

[145,146,149,151,153], the thymol flocculation test [147], and recently particularly for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases [154,155]. However, at least until the recent discovery of the serum 5-nucleotidase [156,157], the determination of serum phosphatase activity afforded the only chemical means for evaluating the patency of the biliary passages, although the bromsulfalein retention and serum cholesterol determination may serve the purpose under certain circumstances. Consequently, the serum alkaline phosphatase determination, for all its limitations, has played a quite unique role in the differential diagnosis of hepatobiliary disorders. The empirical basis for this role is indicated in the next section.

Obstruction of the Extrahepatic Biliary Tract. A compilation from the literature [9,47,48,51,158-162] of data on 150 cases of obstruction of the extrahepatic biliary tract by neoplasm (carcinoma of the head of the pancreas, common bile duct or gallbladder) indicates that in 141 cases (94 per cent) serum alkaline phosphatase levels were in excess of 10 Bodansky units or 30 King-Armstrong units. It is under these circumstances of usually complete and protracted occlusion of the common bile duct that a pronounced increase in the serum enzyme most consistently occurs. The absence of a marked rise in serum alkaline phosphatase activity when pronounced jaundice is present argues strongly against occlusion of the extrahepatic biliary tract by neoplasm.

In calculus or stricture of the common bile duct, obstruction is more apt to be incomplete and/or intermittent, and increased serum alkaline phosphatase levels are less regular in occurrence and likely to be less pronounced. In a compilation of data on 150 cases from the literature [9,47,48,51,158-162], the serum alkaline phosphatase activity was more than 10 Bodansky units or 30 King-Armstrong units in 114 (76 per cent). In occasional cases the finding of an unexpectedly high serum enzyme level, often in the absence of appreciable hyperbilirubinemia, may provide the first clue to diagnosis in obscure problems arising from a "silent" stone in the common bile duct; in this connection, the determination is of value also postoperatively, when it is uncertain whether choledocholithiasis has been completely corrected [51,56,163-166].

Of the total of 300 cases of obstruction of the extrahepatic biliary tract due to neoplasm, stone or stricture, the serum alkaline phosphatase

activity exceeded 10 Bodansky units or 30 King-Armstrong units in 255 (85 per cent). Popper and Schaffner [144] have compiled the data of virtually the world literature and report that of 267 cases of malignant biliary obstruction, in 3 per cent the serum alkaline phosphatase was normal, in 21 per cent "mildly elevated" and in 76 per cent "markedly elevated." The corresponding distribution of 225 cases of benign biliary obstruction was 3.9, 63.4 and 32.7 per cent.

Obstruction of the Intrahepatic Biliary Tract; Intrahepatic Cholestasis. In the final analysis, intrahepatic obstruction to the outflow of bile, or of individual components of bile, occurs at one or another level of the hepatic structure (cells or biliary channels) in virtually all forms of jaundice save those due to extrahepatic overproduction (as in hemolytic jaundice) or to occlusion of the extrahepatic biliary tract [144,167]. Moreover, in many circumstances there is no sharp dividing line between intracellular and extracellular sites of impediment to free bile flow; there are many indications of impingement to some extent upon the fragile bile capillaries where there is hepatocellular injury on the one hand, and of sympathetic hepatocellular damage with injury to the intrahepatic (as well as extrahepatic) biliary radicles on the other. Nevertheless, it is useful to differentiate primary causes and sites of the dislocation of bile flow. The concept of obstruction of the intrahepatic biliary tract [51,57], which attempts to do this, is essentially a functional hypothesis of a state expressed physiologically by increased serum alkaline phosphatase activity (with or without hyperbilirubinemia) and anatomically by its structural counterpart, intrahepatic cholestasis [168], in the absence of obstruction of the extrahepatic biliary tract. Regurgitation of bile is implied, caused either by interposition of structural elements within the liver substance or by a change in the "permeability" of the bile canaliculi without a visible site of obstruction inside or out of the liver. Four groups of hepatobiliary disorders classified within the category of obstruction of the intrahepatic biliary tract will be considered in this context.

The first consists of such space-occupying lesions as hepatic metastasis and abscess. Increased serum alkaline phosphatase activity has long been known to be a common accompaniment of neoplasms metastasizing to the liver [30,51,55], and Meranze, Meranze and

Rothman [55] early stressed the value of the determination in the detection of hepatic metastases, but only recently has this received wide notice [164,166,169-178]. Incipient metastases to the liver frequently fail to elicit a rise in serum alkaline phosphatase activity, occasionally even extensive metastatic involvement of the liver fails to do so, nevertheless the over-all correlation between the serum enzyme level and the presence (or absence) of hepatic metastases is reputed to be as high as 90 per cent in advanced cases [166,170]. Thus the determination, although not wholly dependable, would seem to be at least as useful as any chemical test available to detect hepatic metastases in patients known or suspected to harbor a neoplasm [164,166,170,172,173,178]. To be sure, elevated serum alkaline phosphatase levels do not distinguish between hepatic secondaries and those to bone with osteoplastic response, but this is not too consequential if the objective is to establish whether or not a primary tumor has spread.

An interesting feature of metastatic involvement of the liver (and also of amebic abscess of the liver, to which the preceding remarks generally apply [51,164]) is the prevalence of "dissociation" of serum alkaline phosphatase and serum bilirubin levels, the latter often being quite normal or but slightly raised; indeed Gibbons [166], citing a large and representative experience, found hepatic metastasis to be by far the most common cause of such dissociation. This dissociation has been projected as an obstacle to acceptance of partial obstruction of the intrahepatic biliary tract by the space-occupying tumor, with bile retention, as an explanation for the enhanced serum alkaline phosphatase activity observed but, for reasons to be considered subsequently, this objection does not seem to be insuperable. It must be conceded, however, that apparent discrepancies between the mass of obstructing tumor and the degree of increase in serum alkaline phosphatase activity occur often enough [179]. On the other hand, a general correlation appears to obtain. For example, O. Bodansky [176] gives a mean of 4.8 Bodansky units when single or several nodules are present in the liver; 8.5 units with numerous nodules; and 12.5 units with more extensive involvement.

A second category of obstruction of the intrahepatic biliary tract comprises the granulomatous and infiltrative diseases of the liver, such as sarcoidosis [56,180-184], tuberculosis [51,56,185,

186], Hodgkin's disease [51,56,187] and amyloidosis [56,179,188]. Although increased serum alkaline phosphatase activity is irregular in occurrence and often of a low order in these diseases, even in some cases with substantial infiltration of the liver, the determination may give the first clue to localization of the abnormality in the liver [56]. In this group of disorders, too, there is apt to be a striking dissociation between the serum alkaline phosphatase activity, when it is markedly increased, and the serum bilirubin level, which is apt to show little or no rise. The problems of interpretation of the dissociation in this context have recently been discussed by Ross, Iber and Harvey [56].

A third category is comprised of drug-induced hepatotoxicity [189,190] of the cholestatic type, first observed following the administration of arsphenamine [57] and subsequently in association with the use of a variety of other drugs [190,191], notably chlorpromazine. Whereas the hepatic injury produced by most hepatotoxic compounds is characterized chiefly by hepatocellular necrosis [144,189,190], and the ensuing jaundice is accompanied by little or no rise in serum alkaline phosphatase activity, the drugs in question elicit a hypersensitivity reaction which particularly affects the hepatobiliary system [57,192] and is characterized by the typical manifestations of obstructive jaundice. The clinical reaction is ushered in by prodromal systemic symptoms of allergic character which are followed, in the overt case, by protracted jaundice accompanied by light stools, dark urine and pruritus, and easily confused with obstruction of the extrahepatic biliary tract if a history of drug administration is not obtained. A characteristic feature is elevation of the serum alkaline phosphatase and often of the serum cholesterol, with little or no reaction of the flocculation tests although a modest rise in serum glutamic oxaloacetic transaminase activity often occurs. In one report the serum alkaline phosphatase activity in patients after the administration of arsphenamine was mostly in the range of 20 to 40 Bodansky units [57]; in a representative experience of twenty-two cases of chlorpromazine jaundice [193] the increase in serum enzyme was somewhat less pronounced, up to 36 King-Armstrong units. The determination of serum alkaline phosphatase activity has been suggested as a means of early detection of this type of drug reaction in patients receiving chlorpromazine [194].

In such cases liver biopsy reveals marked intrahepatic cholestasis usually with cellular periportal infiltration [57,144,168,190], morphological indications of the regurgitation of bile that doubtless is responsible for the hyperbilirubinemia and increased serum alkaline phosphatase activity. The liver parenchyma, on the other hand, appears to be well preserved, in relation to the degree of hyperbilirubinemia and the extent of intrahepatic cholestasis present [57], but more detailed examination usually reveals some evidences of hepatocellular injury [168,190,195] and a distinct reduction in the microvillous lining of the biliary canaliculi [190]. The nature and primary site of obstruction of the intrahepatic biliary tract in these cases is still obscure. It has been suggested [57] that the "hypersensitivity" in this type of drug response reflects a metabolic error (enzyme lack) in degradation of the drug by the liver cell, as a consequence of which the integrity of the finer biliary radicles is compromised, with resultant increased "permeability" of the bile capillaries and regurgitation of bile; how much of a role the obstructive effect of bile inspissation, stasis and thrombus formation may play is a moot question.

The fourth category of obstruction of the intrahepatic biliary tract to be considered at this point is biliary cirrhosis not related to mechanical occlusion and/or infection of the extrahepatic biliary channels. This may follow the cholestatic form of viral hepatitis (cholangiolitic cirrhosis) [196-198] or drug hepatotoxicity [199-201], or may arise apparently *de novo* (primary biliary cirrhosis [202,204]). The morphological changes in the liver in these disorders resemble, in all essentials, those accompanying protracted obstruction of the extrahepatic biliary tract which, however, is patent. A marked increase in serum alkaline phosphatase activity, often to levels of 30 to 100 Bodansky units, uniformly characterizes these cases, usually in association with striking elevation of the serum cholesterol, sufficient in many instances to induce xanthomatosis. The degree of accompanying hepatocellular necrosis and regeneration is variable.

Viral Hepatitis, Cholangiolitic Hepatitis, Infectious Mononucleosis. Although the percentages in individual reports vary appreciably, the general consensus is that the majority of patients with viral hepatitis have distinctly lesser increases in serum alkaline phosphatase activity, irrespective of the degree of hyperbilirubinemia, than are

usually encountered in obstruction of the extra- or intrahepatic biliary tract. Thus in a compilation of 372 cases from the literature [47,48,51,158,160,161,205,206], the values were below 10 Bodansky units or 30 King-Armstrong units in 330 (89 per cent). Other reports of large series [207-211], while not giving detailed data, indicate an incidence of from less than 10 to not more than 20 per cent of cases of viral hepatitis with serum alkaline phosphatase activity in the range prevalent in obstructive jaundice. In their inclusive compilation of more than 500 cases of acute hepatitis from the literature, Popper and Schaffner [144] found 34 per cent of the values cited to be within the normal range, 56 per cent to be "mildly" elevated and 10 per cent to be "markedly" elevated. (Hemolytic jaundice, unless associated with stone in the common duct or parenchymal hepatocellular complications, invariably is accompanied by normal serum alkaline phosphatase levels.)

From the start there has been a sharp divergence of opinion as to the source and significance of the usually modest but sometimes more pronounced increase in serum alkaline phosphatase activity in hepatogenous jaundice. One view, initially championed particularly by A. Bodansky [53,212], assumes an hepatic origin of all or most of the serum enzyme increment. At first the enzyme was considered to derive from leakage of the alkaline phosphatase of injured liver parenchymal cells into the bloodstream but most observers (with some exceptions [179]) have failed to find any proportionality between the extent of hepatocellular necrosis and the degree of rise in serum alkaline phosphatase activity, notably in massive necrosis of the liver. Indeed, if any such correlation exists at all it appears more likely to be a negative one, hence the "hepatogenic" school now for the most part seems to accept the general thesis [213] that the degree of increase in serum alkaline phosphatase activity in jaundice depends upon two factors: the extent to which the functional state of the liver parenchyma is preserved, and the degree of obstruction of the biliary tract. In this view the hepatic and/or cholangiolar [214] cells normally secrete hepatic alkaline phosphatase into the bile, and it is further assumed that in hepatitis there is enhanced retrograde secretion of the hepatic enzyme into the sinusoids, either directly by the more or less intact parenchymal cells or by way of regurgitation through bile

canaliculi. The alternative "retention" theory [45,51, and others], on the other hand, considers that some degree of obstruction of the intrahepatic biliary tract probably occurs even in acute viral hepatitis, due perhaps to impingement upon the bile capillaries, as indicated for example by the presence of slight intrahepatic cholestasis demonstrable histologically in most such cases. In this view such increase in serum alkaline phosphatase activity as occurs is attributable to obstructive retention of the enzyme, which is considered to be derived wholly or in large part from extrahepatic (osseous) sources. These theories will be considered in greater detail subsequently.

In sharp contrast to the garden variety of viral hepatitis is the experience with that occasional form of viral hepatitis characterized by pronounced intrahepatic cholestasis and comparatively little overt hepatocellular necrosis (cholangiolitic hepatitis) [144,167,168,196,198,215-217]. Here the serum alkaline phosphatase activity is quite uniformly increased, within the range typical of obstruction of the extrahepatic or intrahepatic biliary tract; indeed all indications, clinical, laboratory and pathologic, indicate that cholangiolitic hepatitis is in fact a form of obstruction of the intrahepatic biliary tract of viral etiology. As previously indicated, there is evidence [196-198] that cholangiolitic hepatitis may progress, in some instances, to cholangiolitic cirrhosis, which has already been described as characterized by high serum alkaline phosphatase and cholesterol levels.

Involvement of the liver in infectious mononucleosis, only occasionally with associated overt jaundice, is often accompanied by a rise in serum alkaline phosphatase activity [218-224], as noted in 65 per cent of fifty-six cases culled from the literature [144]. Levels well in excess of 10 Bodansky units or 30 King-Armstrong units occur in a substantial proportion of cases [218-224], and (as in cholangiolitic hepatitis) these may compound the difficulties in differential diagnosis. The pathogenesis of the increase in serum enzyme is still obscure but it may be related to the infiltration of the liver by lymphoid cells, since there is some histologic indication of accompanying obstruction of the intrahepatic biliary tract [144,223,224].

Postnecrotic and Laennec's Cirrhosis; Primary Hepatic Neoplasia. In a representative series of thirty cases of non-biliary cirrhosis [51], chiefly Laennec's cirrhosis, the serum alkaline

phosphatase activity was within normal limits or only moderately increased in all but six, in which levels in excess of 10 Bodansky units were obtained (maximum, 20 Bodansky units). This general distribution seems to have been the usual experience. Popper and Schaffner [144] compiled from the literature the data in approximately 250 cases of non-biliary cirrhosis without jaundice and found 44 per cent of the serum alkaline phosphatase values to be within normal limits, 56 per cent to be "mildly" elevated, none to be "markedly" increased; in some 300 cases of non-biliary cirrhosis with jaundice, about 27 per cent gave values within normal limits, 65 per cent were "mildly" increased and 8 per cent were "markedly" elevated. The determination of serum alkaline phosphatase activity has not proved to be of value in the diagnosis of cirrhosis, and the pathogenesis of the increased serum enzyme levels (which has been related to regeneration of parenchymal or ductular tissue) is difficult of analysis.

Primary carcinoma of the liver, now seen more frequently as a complication of postnecrotic cirrhosis [225], is irregularly associated with an increase in serum alkaline phosphatase activity beyond the levels attributable to the underlying cirrhosis [51,225-227]; the determination cannot, however, be relied upon to detect neoplastic change.

ORIGIN AND FATE OF THE SERUM ALKALINE PHOSPHATASE(S) IN MAN

The several enzymes normally present in the blood plasma derive from the tissues (or from the formed elements of the blood) where they are spatially distributed in the mitochondria, endoplasmic reticulum (the microsomal fraction, particularly rich in alkaline phosphatase), nucleus and other components of the cell architecture [22,228]. Of the many hundreds of intracellular enzymes which have been identified, however, only a handful can be detected in the blood plasma of normal subjects, fewer still are in appreciable concentration. Thus the great preponderance of enzymes remain within the confines of cells, where they carry out their metabolic functions, unless released into the circulatory fluids by cellular disorganization or by a specialized secretory apparatus.

Cell injury or aging may result in abnormal leakage of intracellular enzymes into the extra-

cellular fluid, at the expense of the tissue enzyme content, which doubtless accounts for the increased number and quantity of circulating enzymes encountered in a variety of diseases. In the case of enzymes widely and abundantly distributed in the tissues, such as those concerned with the several sequences of glucose utilization, liberation into the extracellular fluid may occur in a number of different disorders; in the case of more specialized distribution of an enzyme, such as prostatic acid phosphatase or hepatic glucose-6-phosphatase, increased serum enzyme activity has a more specific diagnostic connotation. But although the alkaline phosphatases are widely distributed in the tissues, notably in the bone, calcifying cartilage, intestinal mucosa, liver and kidney, according to the results of both tissue extraction and histochemical methods [19,229], only in certain diseases of the skeletal and hepatobiliary systems is the serum alkaline phosphatase activity appreciably enhanced, as already noted.

It has been pointed out [230] that the osteoblast (chondrocyte) occupies an exceptional position in respect to the alkaline phosphatase of the blood plasma. The osteoblast (chondrocyte) is functionally, in effect, a one-cell secretory gland: it does not itself calcify but the intact cell normally excretes alkaline phosphatase into the circumambient fluid, presumably for participation in the extracellular processes of bone formation. It should also be appreciated that the aggregate number of osteoblasts and chondrocytes in the body is equivalent in mass to a sizable organ. It goes without saying that hepatic cells are, of course, also strategically situated, in respect to secretion of alkaline phosphatase into the bile.

Whatever the tissue source(s) of the circulating alkaline phosphatase, the levels in the blood plasma of normal subjects, and the equilibria reached in disease, are in a dynamic steady state [237] which reflects the net resultant of the rate at which the enzyme enters the circulating fluids, and the rate of its disposal by excretion, degradation or deposition in the tissues. None of these rates has, as yet, been precisely measured. Moreover, dislocation of the enzyme blood level by a change in one parameter, for example the rate of excretion of the enzyme in the bile, affects the other parameters as a new steady state is established. An illustration of the range of adjustment which thus takes place is given by the

variation in serum alkaline phosphatase activity—from 9.6 to 113 Bodansky units—in forty-five patients with neoplastic obstruction of the common bile duct, and with consistently acholic stools [57]; i.e., all having complete and protracted closure of the principal excretory channel. It follows that in experiments involving drastic operations on the liver, for example, and profoundly affecting the whole organism, consideration should be given not only to direct effects upon excretion of the enzyme in the bile but also to indirect effects on production of the enzyme by osteoblasts, due to cessation of growth and like factors. Starvation and grossly inadequate diets [232,233] similarly may affect growth and enzyme production, as may a host of other factors. Under these circumstances induction of what seems to be a single experimental variable may, in fact, initiate a complex chain of events influencing the level of serum alkaline phosphatase activity. Interpretation of the results consequently is apt to be oversimplified.

The plasma concentration of bilirubin glucuronide, and of other plasma components appearing in the bile, also reflects a steady state, each regulated by its own rate of biosynthesis and routes of disposal. When the common excretory channel, the biliary tract, is blocked, each such component re-establishes equilibrium at its own level, determined by its individual regulatory apparatus. It should therefore not be surprising that the new steady states of the several components (bilirubin glucuronide, alkaline phosphatase, cholesterol esters, bile acids, etc.) may be re-established at quite unequal levels, a phenomenon rather ingenuously termed "dissociation." Thus in partial obstruction of the intrahepatic or extrahepatic biliary tract the serum alkaline phosphatase activity may be substantially increased whereas the serum bilirubin glucuronide remains within normal limits or is but slightly elevated, an instance of dissociation which has given rise to much speculation, and is considered by some to disqualify the "retention" theory. One obvious factor involved is that whereas bilirubin glucuronide readily escapes in the urine under these conditions, the enzyme protein cannot pass through the glomerular membrane, hence is more rapidly impounded in the blood [41,234]. Other explanations have been offered, based on the possibility of graded liver cell injury [212,235], differential retrograde bile pressures [165], and the like. In the dog this dissociation is even more striking

than in man. Such is the capacity of the dog kidney to clear bilirubin glucuronide that even complete occlusion of the common bile duct produces little jaundice whereas the serum alkaline phosphatase activity rapidly rises to spectacular levels (some 500 King-Armstrong units [42] or 300 Bodansky units [52]), far greater than ever encountered in comparable states in man.

Such species differences may introduce confusion in the experimental approach to the origin and fate of the serum alkaline phosphatase(s). In the dog, so great is the potential for increase in the serum enzyme when the patency of the intrahepatic or extrahepatic biliary tract is even slightly compromised that it is difficult to decide whether the rise accompanying hepatocellular damage (and this is apt to be substantial in comparison to levels in man) should be attributed to liver cell injury, as often has been inferred, or to the minor impingement upon the finer biliary radicles with which such "hepatitis" almost inevitably is associated. In the cat, on the other hand, ligation of the common bile duct results in more distinct hyperbilirubinemia, but in only slight enhancement of serum alkaline phosphatase activity, far less than that in the dog or man [236-240]. In this species, unlike dog and man, the urine (as well as the bile) normally contains appreciable quantities of alkaline phosphatase [237,239,240], implying passage of the enzyme through the intact kidney. This is the one species also in which histochemical examination regularly reveals alkaline phosphatase activity of the glomerulus [229,239,241]. Since urine alkaline phosphatase in the cat tends to increase transiently after ligation of the common bile duct [237,239,240], it seems likely that the enzyme in the serum escapes in the urine and hence does not accumulate markedly; the evidence on this point, however, is inconclusive [237,239]. The status of the rat is different again. In this species the serum alkaline phosphatase normally is maintained throughout life at exceptionally high levels (of the order of 60 to 80 Bodansky units), appears to be quite independent of the rate of growth, and is unusually susceptible to variations in diet. For these and other reasons considerable attention has been given to the possibility of an intestinal origin of the enzyme in this species [242-247].

A question about serum alkaline phosphatase levels which has been raised from time to time is whether the alterations encountered in disease

and under certain experimental conditions may be due not to changes in concentration of the enzyme, but to the presence of activators or inhibitors affecting enzyme activity [248,249]. The point is appropriate because, while enzyme concentrations can be estimated [250], the conventional methods employed measure only activity. Moreover, it has been demonstrated that, in appropriate molarities, various metal cations and amino acids activate alkaline phosphatase [250,251] and taurocholate, amino acids in higher concentrations and serum albumin inhibit the enzyme [252,253]. Nevertheless, these effects appear to be minor *in vivo*, and the consensus, based upon relevant experiments [254-258], is that the alterations in serum alkaline phosphatase activity encountered in disease states reflect bona fide changes in enzyme concentration.

"Retention" Theory Versus "Hepatogenic" Theory. Only two of the many formulations of the origin and fate of the serum alkaline phosphatase in man will be considered here in any detail. The first assumes the plasma alkaline phosphatase to be almost exclusively of osseous origin and the role of the liver to be solely excretory, by way of the bile; if the biliary outlet is anywhere compromised, retention of the (extrahepatically derived) alkaline phosphatase in the plasma occurs. Stipulation of the osseous origin of the serum enzyme is based on the abundance of the enzyme in the skeleton, its regular secretion by the osteoblast (chondrocyte) into the extracellular fluid, the rise in serum enzyme activity in normal growth and in skeletal disorders overproductive of bone, and the decline in the serum enzyme level in hypophosphatasia, in which the bone enzyme is deficient. Retention of serum alkaline phosphatase, when the biliary tract is obstructed, is predicated on the absence of the enzyme from the urine in man and the high alkaline phosphatase activity of the bile. It is highly probable, although not quantitatively fully established, that the biliary tract is by far the most important excretory route of the enzyme [259]; such being the case, obstruction of the intrahepatic or extrahepatic biliary channels would be expected to result in retention of the enzyme in the blood, irrespective of its origin. The mechanism by which this regurgitation takes place is indicated histochemically by intense alkaline phosphatase staining of the distended bile canaliculi, which are seen to extend well up to the sinusoidal barrier, follow-

ing obstruction of the common bile duct [240,261-264]. Direct confirmation of Eppinger's concept of obstructive regurgitation of bile through distended and ruptured bile capillaries, with retrograde flow into the sinusoids, is afforded by intra vitam studies using sodium fluorescein as tracer, notably by Hanzon [264,265]. Particulate matter of the bile, however, is returned via the parenchymal cells [354].

The second formulation assumes that a significant proportion of the plasma alkaline phosphatase normally is of hepatogenic origin, and that the hepatobiliary system, when it is disordered, contributes wholly or in large part to the increase in plasma alkaline phosphatase activity which then ensues. (This hepatogenic mechanism does not attempt to account for the increased serum alkaline phosphatase activity of skeletal disorders or for disposal of the osteogenic enzyme when the excretory biliary channels are blocked.) The increase in serum alkaline phosphatase activity occurring in hepatobiliary disease is ascribed to overproduction of the enzyme by liver parenchymal and/or ductular (cholangiolar) cells [214], and/or to obstruction of biliary tract excretion of the enzyme produced and secreted by the liver cells at the normal rate. Another school of thought considers the increased serum phosphatase activity in hepatocellular disease to be due to leaching out of the enzyme from injured liver parenchymal cells but if this last were the case the serum alkaline phosphatase should rise in parallel with the degree of parenchymal liver cell injury and of bilirubinemia, in the manner characteristic of such hepatocellular enzymes as glutamic oxaloacetic and glutamic pyruvic transaminases [154,155,266], glucose-6-phosphatase [267] and lactic dehydrogenase. This clearly does not occur. The divergence is most striking in acute massive necrosis of the liver, in which the trifling rise in serum alkaline phosphatase activity contrasts strangely with the marked serum elevations of other hepatocellular enzymes; a difference not acceptably explained, as has been proposed, by rapid exhaustion of the hepatocellular supply of alkaline phosphatase since no clear correlation with serum enzyme levels can be made out at any stage of progressive hepatocellular failure. In fact, histochemical examination reveals more alkaline phosphatase within the liver parenchymal cell in hepatitis than is observed normally or in obstructive jaundice, as Sherlock [268] and others have

stressed. It should be appreciated in this connection that the normal liver parenchymal cell is really not particularly rich in alkaline phosphatase in comparison with the intracellular content of many other enzymes, as shown by standard histochemical, tissue extraction, or the more refined subcellular fractionation methods [240,260-263,269-271].

The seemingly complete indifference of the serum enzyme level to the state of the hepatic parenchyma makes it difficult, in fact, to understand how the liver cell can be chiefly instrumental in either the secretion or excretion of alkaline phosphatase, whether intrahepatically or extrahepatically derived. It has been suggested [51] that, unlike bilirubin, the serum alkaline phosphatase may be, at least in part, abstracted from the sinusoids directly into the bile capillaries, thus bypassing the polygonal cells. This speculation presumes that the bile canaliculi normally are in functional communication with the sinusoids, a thesis that has received increasing anatomical support in recent years [272-274], although it is not generally accepted by hepatologists. But even if it were conceded that extrahepatically derived alkaline phosphatase may pass through the liver by some such shunt route, it would be necessary to predicate some secretory (cellular) process [355] for transfer of the enzyme molecules across the sinusoidal barrier. Relevant aspects of these and other complexities of bile secretion have recently been discussed by Brauer [275]. Until the routes and mechanisms of alkaline phosphatase transfer through the liver can be clarified, complete resolution of the problems of the origin and fate of the enzyme in man must remain in abeyance.

Results of Experimental Modification of the Hepatobiliary System. Of the experimental evidence offered pro and con in these and other formulations, much is subject to conflicting interpretation which it is not possible at present wholly to resolve. Nevertheless, the data so obtained are of considerable interest and importance.

Perhaps the most significant results are those of complete hepatectomy in the dog, an approach that proved in a vexing analogous problem to be decisive in establishing the chiefly extrahepatic source of bile pigments in the mammal [276]. As shown by Armstrong and Banting [277], and subsequently by others [278-281], total hepatectomy in the dog is followed not by a decrease but by sustained or

increased serum alkaline phosphatase activity. Moreover, an increase in serum enzyme occurs in this species also after removal of the small or large intestine, spleen, pancreas, kidneys, indeed of all the abdominal viscera [277-279,282], signifying that neither the liver nor any of the other abdominal organs can be considered to be the sole or major source of serum alkaline phosphatase in the dog; however, minor contributions by these viscera are by no means excluded by these experiments. As would be expected on the basis of the similar experience with serum bilirubin after total hepatectomy in dogs, the rise in serum alkaline phosphatase activity is a relatively modest one, presumably because production of the enzyme by osteoblasts in the moribund hepatectomized animal comes virtually to a halt. The increase in serum enzyme following ligation of the common bile duct (after which dogs hardly turn a hair) usually is much more striking. It has been argued that, since both operations result in complete loss of biliary excretory capacity, the increase in serum alkaline phosphatase activity should in both cases be the same, inferring that the discrepancy is due to continued elaboration of the enzyme by the liver left *in situ* after ligation of the common bile duct [280,283]. (This ingenious explanation does not account, however, for the failure of the serum alkaline phosphatase to decline or disappear after total hepatectomy.) After partial hepatectomy in the dog—removal of some 70 per cent of the liver—there was an initial small increase in serum alkaline phosphatase (as after total hepatectomy), followed by a more striking rise in serum enzyme [287]. After removal of about one-third of the liver [284], the serum alkaline phosphatase activity exhibited a slight and transient rise. Removal of 70 per cent of the liver in the rat has been followed consistently by a sharp rise in plasma and liver alkaline phosphatase [285-287].

As already noted, ligation of the common bile duct in the dog produces a rapid and marked increase in serum alkaline phosphatase activity [42,52,238,240,280,282,284,288-291]. Ligation of one or more major hepatic ducts in the dog also is followed by an increase in serum alkaline phosphatase activity [163,234,284,288], to levels roughly parallel to the proportion of the total liver drainage so occluded. These results are the experimental counterpart of the elevated serum alkaline phosphatase occurring in man with complete obstruction of the common bile

duct respectively incomplete obstruction of the extrahepatic or intrahepatic biliary tract. They have not led to any decision as to whether the increment in serum enzyme, when the biliary tract is compromised, is of extrahepatic or hepatic origin but they leave no doubt that the bile is the chief medium of excretion of the serum enzyme, at least in the dog.

The reverse experiment, creation of a biliary fistula in the dog [259,284,292,293], at first gave some results that seemed to be quite inconsistent with the retention theory, *viz.* rising serum alkaline phosphatase levels in the face of apparently free flow of bile through the fistula. Dalgaard's critical analysis [259] makes it clear, however, that this occurs only when the fistulous tract is infected or otherwise obstructed; when the bile is freely discharged through a clean and well maintained fistula the copious bile flow is usually rich in alkaline phosphatase and there is little or no increase in the serum enzyme activity, *i.e.*, there is little or no retention of the enzyme. This construction of the events corresponds closely to what is observed clinically in man [57]: The creation of a bile fistula postoperatively in obstructive jaundice is followed by a decline in serum alkaline phosphatase activity when there is free drainage of bile. Failure of the serum enzyme to fall, or a secondary rise, with or without hyperbilirubinemia, implies either that the obstructing nidus has not been completely removed or that bile drainage is impaired by infection or other cause. The usefulness of the determination of serum alkaline phosphatase in this connection is now well substantiated.

The role of the bile in the excretion of alkaline phosphatase in the dog has been studied also by serial estimations of bile and plasma enzyme levels after intravenous injection of alkaline phosphatase. These experiments uniformly indicate that the enhanced serum alkaline phosphatase activity so produced results in an increase in the concentration of the enzyme excreted in the bile, of a degree and at an excretory rate more or less dependent upon the quantity of alkaline phosphatase injected; but in no case could more than a fraction of the injected enzyme be recovered in the bile. When relatively small quantities of enzyme were infused, in the form of phosphatase-rich serum [294-297], as often as not the recipient dogs' serum alkaline phosphatase activity rose to levels considerably higher than could be accounted for by the

amount of enzyme injected. This was explained [296,297] by "activation" of the alkaline phosphatase [248,249,298] but, as already indicated, there is no acceptable basis for this interpretation [254-258]; it seems more likely that the injections in some way caused minor damage to the liver and/or intrahepatic biliary tract. When larger quantities of enzyme were delivered, in the form of purified intestinal mucosa preparations, the clearance of the increased serum alkaline phosphatase through the liver was more rapid [299,300], and the enzyme concentration of the excreted bile (collected through a fistula) and of the liver parenchyma rose markedly [300]. Injection of the enzyme into hepatectomized dogs, on the other hand, led to a progressive rise in serum alkaline phosphatase activity to extremely high levels [299], whereas nephrectomized dogs cleared the injected enzyme at the normal rate [299].

Finally, it has been possible to reproduce in the dog, by administration of various hepatotoxic drugs [212,240,284,288,301,302, and others] and by inoculation of leptospira [284], the relatively modest rises in serum alkaline phosphatase activity observed in hepatitis and in drug-induced hepatocellular damage in man. Much the same difficulty that besets explanation of the findings in man cloud interpretation of the experimental findings in the dog, and opinion is divided as to whether the increase in serum enzyme under these circumstances arises *de novo* from injured parenchymal cells or is a consequence of impaired passage of extrahepatically derived alkaline phosphatase through the altered liver cells or contiguous bile canaliculi. It should be noted that, in view of the extraordinarily high serum alkaline phosphatase activity of the dog with complete obstruction of the excretory biliary channels, minor impingement upon the bile canaliculi would probably suffice to account for the moderate increases in serum enzyme associated with induced hepatitis.

Results of More Direct Attempts to Identify the Plasma Alkaline Phosphatase(s) in Relation to Tissue Phosphatases. A more direct assault upon the problem of the origin of circulating alkaline phosphatase is possible by defining the properties of the enzyme in the plasma in relation to the corresponding properties of the several tissue alkaline phosphatases. One method which has been employed depends upon the comparative degree of inhibition of enzyme activity by a wide variety of compounds [303]. It is not possible to

prove identity by this means (the uncertainties encountered are like those in establishing the purity of an enzyme protein), particularly in so complex a system of proteins as blood plasma represents, but useful information can be derived by establishing distinct differences. The first such experiments indicated that the alkaline phosphatase of normal serum resembled bone alkaline phosphatase in respect to inhibition by oxalate but differed from liver alkaline phosphatase [304]. Cyanide, which markedly suppresses bone alkaline phosphatase activity [305,306], has been variously reported to inhibit serum alkaline phosphatase 80 to 90 per cent in normal man [230], and not to inhibit appreciably [307,308]; however, there was agreement as to the marked inhibition of the increased serum alkaline phosphatase activity of obstructive jaundice [230,308], inhibition of about the same degree as occurs in skeletal disorders characterized by hyperphosphatasemia [230]. The alkaline phosphatase activity of the serum of normal man and of patients with Paget's disease is markedly suppressed by bile acids, which inhibit bone and kidney alkaline phosphatase but have no effect on the enzyme present in intestinal mucosa [252].

Fractionation of the serum alkaline phosphatase by anion exchange resins in two patients with increased serum enzyme levels due to neoplasia with metastases yielded a single peak of alkaline phosphatase activity in one instance, in which a Dowex-2 column was employed [309], and two peaks (one much broader than the other) in the second instance using a DEAE-cellulose column [310]. Paper electrophoresis of serums with increased alkaline phosphatase activity, whether related to skeletal or hepatobiliary disorders, revealed a higher than normal alpha-2-globulin peak [5,7] and a second peak in the alpha-1-globulin region [5,7]. In some hands [311], however, the increase was reported solely in the zone of beta-1- and beta-2-globulins (? tailing); a similar finding with starch gel electrophoresis has been recorded in one patient with biliary cirrhosis and in another with Paget's disease [10]. Keiding [11], using starch block electrophoresis, noted a marked increase in the alkaline phosphatase activity of the serum beta-globulin peak in Paget's disease and in carcinoma with bone metastases; sometimes an increase also in the alpha-2-globulin peak (but not in the alpha-1-globulins). In obstructive jaundice, on the other hand, there was no increase in phosphatase activity in the beta-

globulin region, only in the zone of alpha-2-globulins and often slightly also in the zone of alpha-1-globulins. Rosenberg's starch electrophoresis studies [9] gave more consistent results. In Paget's disease and prostatic carcinoma with osteoplastic metastases the excess alkaline phosphatase activity uniformly appeared as an enlarged alpha-2-globulin peak, with little or no increase in the area of alpha-1-globulins and only nondescript "tailing" in the beta-globulin zone. In cases of obstructive jaundice, with a mean twelvefold normal serum alkaline phosphatase level, about five-sixths of the total enzyme activity appeared in the enlarged alpha-2-globulin peak (presumably corresponding to bone phosphatase) and the remainder as a now readily discernible alpha-1-globulin peak. (Both alpha-2 and alpha-1 enzymes were almost completely inhibited by 10^{-2} N sodium cyanide and were not sensitive to sodium fluoride.) The serums of patients with viral hepatitis and moderately increased alkaline phosphatase activity yielded somewhat higher alpha-2-globulin curves, without any detectable change in alpha-1-globulin activity.

Of particular interest are the results of efforts to apply immunochemical methods to the fractionation of serum alkaline phosphatase(s) [312,313]. Schlamowitz and O. Bodansky [313] prepared purified human bone and intestinal mucosa alkaline phosphatase antigens, and with antisera to these were able to remove presumably related proportions of the total serum alkaline phosphatase activity, with some uncertainties due to incomplete and cross precipitation. In three normal human subjects approximately 40 to 59 per cent of the total serum alkaline phosphatase activity could be identified as "bone" phosphatase; 28 to 39 per cent did not react to both anti-bone and anti-intestine phosphatase, hence was unaccounted for. In patients with elevated serum alkaline phosphatase levels due to carcinoma with osteoplastic metastases to bone, metastases to liver, or invasion and obstruction of the common bile duct, "bone" phosphatase constituted 75 to 93 per cent of the total serum alkaline phosphatase activity, and only 6 to 22 per cent was unaccounted for by combined precipitation with anti-bone and anti-intestine phosphatase.

ROLE OF "NON-SPECIFIC" ALKALINE PHOSPHATASES IN METABOLISM

As recently stated [314], "a discussion of (the metabolic role of alkaline) phosphatase is

one of the most embarrassing subjects for a biochemist, and . . . the discussion is not likely to be very useful." Although known since 1912 [315] to be widely distributed in animal tissues (unlike the acid phosphatases, non-specific alkaline phosphatases apparently do not occur in plants, yeasts or bacteria [22]), the place of these enzymes in cellular metabolism remains uncertain. It has been variously suggested that the non-specific alkaline phosphatases are concerned with the biosynthesis of fibrous proteins ([316]; but see [317-319]), mucopolysaccharides [320] and numerous other compounds; that they may serve as regulators of intracellular phosphate concentration [314]; even that they may be artefacts [327]. The evidence on all points is inconclusive.

The role of alkaline phosphatase specifically in bone formation has been the subject of especially intensive investigation, the results of which have been summarized in a number of reviews [19-25, 33, 58-62, 303, 322-330]. The association in time and place of alkaline phosphatase in osteoblasts and chondrocytes with the processes of bone formation in normal skeletal growth, bone repair and abnormal bone proliferation, which so impressed Robison [24], has been substantiated and amplified by more recent studies [326, 330-333]. Moreover, the disturbance in skeletal development when bone is deficient in alkaline phosphatase, as in hypophosphatasia [134-139], adds corroborative evidence to the importance of the enzyme in osteogenesis. Nevertheless, the precise part played by skeletal alkaline phosphatase in bone formation remains obscure. Robison's first and second approximations [24] have proved to be inadequate; the preparative role of phosphorylative glycogenolysis in endochondral calcification [322, 334-337] has not been carried in man beyond the synthesis of high-energy phosphorus compounds available as sources of energy, phosphorus, or both; the formation of matrix fibrous proteins through the agency of alkaline phosphatase [58, 325, 338, 339] has not been established; the possible role of alkaline phosphatase as a phosphotransferase in the synthesis of phosphoric esters serving as substrates for alkaline phosphatase or as complexing agents [322, 340-344] remains speculative. And if, as has been suggested as a result of studies on hypophosphatasia, phosphorylethanolamine is a natural substrate for alkaline phosphatase [137, 140], the role of the enzyme in bone formation is obscure indeed for the present.

CONCLUDING REMARKS

It is apparent from what has gone before that distinct increases in serum alkaline phosphatase activity occur in man solely in skeletal diseases characterized by excessive production of alkaline phosphatase by osteoblasts or chondrocytes; in disorders of the hepatobiliary system particularly when there is obvious impediment to the flow of bile in the extrahepatic or intrahepatic biliary tract; and in diseases, such as neoplasia, which may involve the bones, the liver, or both.

There can be no reasonable doubt that the increase in serum alkaline phosphatase activity characteristic of normal growth and the relevant disorders of bone are of skeletal origin. The properties of the enzyme increment, which appears in the blood plasma under these circumstances, are not presently distinguishable from those of the enzyme which comprises by far the largest part of the total serum alkaline phosphatase activity in normal man. For this and other reasons already set forth there would appear to be sufficient warrant for the assumption that the major component of the normal plasma alkaline phosphatase(s) is of osseous origin; indeed, with the possible exception of the liver, this is the only source of the plasma enzyme in man for which there is acceptable evidence.

There is also substantial support for the view that the hepatobiliary system provides the chief excretory mechanism, by way of the bile, for disposal of the plasma alkaline phosphatase(s). Interference with this excretory route may therefore justifiably be supposed to result in retention of the plasma enzyme. If it be further conceded that the plasma enzyme is indeed largely derived from bone, it follows that the increase in serum alkaline phosphatase activity occurring in obstruction of the biliary tract is due for the most part to retention of skeletal alkaline phosphatase. This conclusion is in fact supported by the experience of most investigators, who have been unable to distinguish the properties of the serum enzyme increment in obstructive jaundice from those of the serum enzyme increment in skeletal disorders, or from those of the major alkaline phosphatase component of normal blood plasma.

This interpretation plausibly links the increase in serum alkaline phosphatase activity in normal growth and in various bone diseases (overproduction of the enzyme) with that encountered in hepatobiliary disorders (retention of the enzyme), and sufficiently accounts for the

absence of any significant rise in serum enzyme activity in circumstances other than those affecting the skeletal or hepatobiliary systems. A disputatious point remains, however. Many investigators have enthusiastically embraced the thesis that the liver normally contributes significantly to the normal complement of serum alkaline phosphatase(s) and that the rise in serum alkaline phosphatase activity in hepatobiliary disorders is wholly or largely of hepatic origin. If the preceding premises are correct, this view must be regarded with reserve; the evidence offered for it is, moreover, inconclusive when considered critically in broad perspective. Nevertheless, it has not been demonstrated that a minor proportion of the normal serum alkaline phosphatase activity does not derive from the liver, and there is some experimental support for an apparently small hepatic contribution to the serum enzyme when the extrahepatic or intrahepatic biliary channels are compromised. Certainly there are apparent inadequacies in the retention theory, but whether these are attributable to deficiencies in the theory or in its application is not altogether clear.

The eventuality of minor contributions to the total serum alkaline phosphatase activity from tissue sources other than bone and liver also has not been excluded. The recent discovery that platelet acid phosphatase [345] is liberated into the serum during clotting [346,347] has reawakened interest particularly in the possibility that leukocyte alkaline phosphatase [348-352] may, under some circumstances, add to the enzyme activity of blood plasma [349,350,353].

Finally, there is the contentious question of the usefulness of the serum alkaline phosphatase determination in the differential diagnosis of hepatobiliary diseases. This is certainly limited by the overlap with skeletal disorders; the overlap between hepatogenous and obstructive jaundice (particularly when there is significant obstruction of the intrahepatic biliary tract in the former and incomplete and/or intermittent obstruction of the biliary tract in the latter); the failure to distinguish obstruction of the intrahepatic from that of the extrahepatic biliary tract (which may lead to serious error); and the failure to differentiate benign from malignant occlusion of the extrahepatic biliary tract. Despite all these limitations, however, the determination remains the most sensitive available chemical criterion of impingement upon

the extrahepatic or intrahepatic biliary tract, and as such would seem to merit, when employed in the proper context, the wide usage which it presently enjoys.

REFERENCES

1. MARTLAND, M., HANSMAN, F. S. and ROBISON, R. The phosphoric-esterase of blood. *Biochem. J.*, 18: 1152, 1924.
2. MARTLAND, M. and ROBISON, R. Possible significance of hexosephosphoric esters in ossification. VI. Phosphoric esters in blood-plasma. *Biochem. J.*, 20: 847, 1926.
3. GUTMAN, A. B. and GUTMAN, E. B. "Acid" phosphatase activity of the serum of normal human subjects. *Proc. Soc. Exper. Biol. & Med.*, 38: 470, 1938.
4. Enzymes and Enzyme Systems. Their State in Nature. Edited by Edsall, J. T. Cambridge, Mass., 1951. Harvard University Press.
5. TALEISNIK, S., PAGLINI, S. and ZEITUNE, V. Localisation de la phosphatase alcaline du sérum dans les fractions protéiniques séparées par électrophorèse. *Compt. rend. Soc. de biol.*, 149: 1790, 1953.
6. BAKER, R. W. R. and PELLEGRINO, C. The separation and detection of serum enzymes by paper electrophoresis. *Scandinav. J. Clin. & Lab. Invest.*, 6: 94, 1954.
7. EISFELD, G. and KOCH, E. Das Verhalten der alkalischen und sauren Serumphosphatase des Menschen bei der Papierelektrophorese. *Ztschr. ges. inn. Med.*, 9: 514, 1954.
8. WOLFSON, W. Q. Location of alpha-2-globulin by demonstration of alkaline phosphatase during paper electrophoresis. *Nature, London*, 180: 550, 1957.
9. ROSENBERG, I. N. Zone electrophoretic studies of serum alkaline phosphatase. *J. Clin. Invest.*, 38: 630, 1959.
10. KOWLESSAR, O. D., PERT, J. H., HAEFFNER, L. J. and SLEISINGER, M. H. Localization of 5-nucleotidase and non-specific alkaline phosphatase by starch gel electrophoresis. *Proc. Soc. Exper. Biol. & Med.*, 100: 191, 1959.
11. KEIDING, N. R. Differentiation into three fractions of the serum alkaline phosphatase and the behavior of the fractions in diseases of bone and liver. *Scandinav. J. Clin. & Lab. Invest.*, 11: 106, 1959.
12. FLEURY, P., COURTOIS, J., ANAGNOSTOPOULOS, C. and DESJOBERT, A. Action de phosphatases de types distincts sur différents esters phosphoriques. *Bull. Soc. Chim. Biol.*, 32: 771, 1950.
13. AXELROD, B. Enzymatic phosphate transfer. *Advances Enzymol.*, 17: 159, 1956.
14. KAY, H. D. The phosphatases of mammalian tissues. *Biochem. J.*, 22: 855, 1928.
15. MORTON, R. K. The phosphotransferase activity of phosphatases. II. Studies with purified alkaline phosphomonoesterases and some substrate-specific phosphatases. *Biochem. J.*, 70: 139, 1958.
16. ROSS, M. H., ELY, J. O. and ARCHER, J. G. Alkaline phosphatase and pH optima. *J. Biol. Chem.*, 192: 561, 1951.
17. WACHSTEIN, M. and MEISEL, E. Histochemistry of

- hepatic phosphatases at a physiologic pH, with special reference to the demonstration of bile canaliculi. *Am. J. Clin. Path.*, 27: 13, 1957.
18. ERDTMAN, H. Ueber Nierenphosphatase und ihre Aktivierung. II. *Ztschr. physiol. Chem.*, 177: 211, 1928.
 19. FOLLEY, S. J. and KAY, H. D. The phosphatases. *Ergebn. Enzymforsch.*, 5: 159, 1936.
 20. ALBERS, H. Phosphatasen. In: *Handbuch der Enzymologie*, vol. 1, p. 408. Edited by Nord, F. F. and Weidenhagen, R. Leipzig, 1940. Akadem. Verlag.
 21. ROCHE, J. Phosphatases. In: *The Enzymes. Chemistry and Mechanism of Action*, vol. 1, p. 473. Edited by Sumner, J. B. and Myrbäck, K. New York, 1950. Academic Press.
 22. DIXON, M. and WEBB, E. C. *Enzymes*. New York, 1958. Academic Press.
 23. ROBISON, R. The possible significance of hexosephosphoric esters in ossification. *Biochem. J.*, 17: 286, 1923.
 24. ROBISON, R. The Significance of Phosphoric Esters in Metabolism. New York, 1932. New York University Press.
 25. ROBISON, R. and ROSENHEIM, A. H. Calcification of hypertrophic cartilage in vitro. *Biochem. J.*, 28: 684, 1934.
 26. KAY, H. D. Plasma phosphatase in osteitis deformans and in other diseases of bone. *Brit. J. Exper. Path.*, 10: 253, 1929.
 27. ROBERTS, W. M. Variations in the phosphatase activity of the blood in disease. *Brit. J. Exper. Path.*, 11: 90, 1930.
 28. KAY, H. D. Plasma phosphatase. II. The enzyme in disease, particularly in bone disease. *J. Biol. Chem.*, 89: 249, 1930.
 29. FRANSEEN, C. C. and McLEAN, R. The phosphatase activity of tissues and plasma in tumors of bone. *Am. J. Cancer*, 24: 299, 1935.
 30. GUTMAN, A. B., TYSON, T. L. and GUTMAN, E. B. Serum calcium, inorganic phosphorus and phosphatase activity in hyperparathyroidism, Paget's disease, multiple myeloma and neoplastic diseases of the bones. *Arch. Int. Med.*, 57: 379, 1936.
 31. ROBISON, R. and SOAMES, K. M. The possible significance of hexosephosphoric esters in ossification. II. The phosphoric esterase in ossifying cartilage. *Biochem. J.*, 18: 740, 1924.
 32. GUTMAN, E. B., SPROUL, E. E. and GUTMAN, A. B. Significance of increased phosphatase activity of bone at the site of osteoplastic metastases secondary to carcinoma of the prostate gland. *Am. J. Cancer*, 28: 485, 1936.
 33. KAY, H. D. Phosphatase in growth and disease of bone. *Physiol. Rev.*, 12: 384, 1932.
 34. BODANSKY, A. and JAFFE, H. L. Phosphatase studies. III. Serum phosphatase in diseases of the bone: Interpretation and significance. *Arch. Int. Med.*, 54: 88, 1934.
 35. CORYN, G. Les phosphatases du sang dans le diagnostic des affections osseuses. *Scalpel*, 87: 1301, 1934.
 36. WOODARD, H. Q., TWOMBLY, G. H. and COLEY, B. L. A study of the serum phosphatase in bone disease. *J. Clin. Invest.*, 15: 193, 1936.
 37. WOODARD, H. Q. and HIGINBOTHAM, N. L. The correlation between serum phosphatase and roentgenographic type in bone disease. *Am. J. Cancer*, 31: 221, 1937.
 38. MORRIS, N. and PEDEN, O. D. Plasma phosphatase in disease: a review. *Quart. J. Med.*, 6: 211, 1937.
 39. MÜLLER, J. *Über Phosphatasen in Het Serum*. Amsterdam, 1938. Scheltema and Holkema.
 40. FRANSEEN, C. C., SIMMONS, C. C. and McLEAN, R. The phosphatase determination in the differential diagnosis of bone lesions. *Surg., Gynec. & Obst.*, 68: 1038, 1939.
 41. ROBERTS, W. M. Blood phosphatase and the van den Bergh reaction in the differentiation of the several types of jaundice. *Brit. M. J.*, 1: 734, 1933.
 42. ARMSTRONG, A. R., KING, E. J. and HARRIS, R. I. Phosphatase in obstructive jaundice. *Canad. M. A. J.*, 31: 14, 1934.
 43. GREENE, C. H., SHATTUCK, H. F. and KAPLOWITZ, L. The phosphatase content of the blood serum in jaundice. *J. Clin. Invest.*, 13: 1079, 1934.
 44. AUSTONI, B. and COGGI, G. La phosphatase du plasma dans différentes affections. *Presse méd.*, 42: 1594, 1934.
 45. HERBERT, F. K. The plasma phosphatase in the various types of jaundice. *Brit. J. Exper. Path.*, 16: 365, 1935.
 46. ANDERSON, R. G. The plasma phosphatase in jaundice. *St. Barth. Hosp. Rep.*, 68: 221, 1935.
 47. ROTHMAN, M. M., MERANZE, D. R. and MERANZE, T. Blood phosphatase as an aid in the differential diagnosis of jaundice. *Am. J. M. Sc.*, 192: 526, 1936.
 48. FLOOD, C. A., GUTMAN, E. B. and GUTMAN, A. B. Phosphatase activity, inorganic phosphorus and calcium of serum in disease of liver and biliary tract. A study of one hundred and twenty-three cases. *Arch. Int. Med.*, 59: 981, 1937.
 49. CANTAROW, A. and NELSON, J. Serum phosphatase in jaundice. *Arch. Int. Med.*, 59: 1045, 1937.
 50. SHAY, H. and FIEMAN, P. The value of a combined study of the newer laboratory tests in the differential diagnosis of toxic and obstructive jaundice including blood phosphatase, cholesterol partition, galactose tolerance and glucose tolerance. *Am. J. Digest. Dis.*, 5: 597, 1938.
 51. GUTMAN, A. B., OLSON, K. B., GUTMAN, E. B. and FLOOD, C. A. Effect of disease of the liver and biliary tract upon the phosphatase activity of the serum. *J. Clin. Invest.*, 19: 129, 1940.
 52. BODANSKY, A. and JAFFE, H. L. Phosphatase studies. VIII. Increase of serum phosphatase after bile duct ligation in dog. *Proc. Soc. Exper. Biol. & Med.*, 31: 1179, 1934.
 53. BODANSKY, A. and JAFFE, H. L. Phosphatase studies. IV. Serum phosphatase of non-osseous origin; significance of the variations of serum phosphatase in jaundice. *Proc. Soc. Exper. Biol. & Med.*, 31: 107, 1933.
 54. ALESSANDRI, H., DUCCHI, C. H. and ARANIBAR, E. La actividad fosfatasa del plasma en el diagnóstico diferencial de las ictericias. *Rev. med. de Chile*, 69: 181, 1941.
 55. MERANZE, D. R., MERANZE, T. and ROTHMAN, M. M. Serum phosphatase as an aid in the diagnosis of metastasis of cancer to liver. *Pennsylvania M. J.*, 41: 1160, 1938.

56. ROSS, R. S., IBER, F. L. and HARVEY, A. M. The serum alkaline phosphatase in chronic infiltrative disease of the liver. *Am. J. Med.*, 21: 850, 1956.
57. HANGER, F. M., JR. and GUTMAN, A. B. Postarsphenamine jaundice apparently due to obstruction of intrahepatic biliary tract. *J. A. M. A.*, 115: 263, 1940.
58. MOOG, F. The physiological significance of the phosphomonoesterases. *Biol. Rev.*, 21: 41, 1946.
59. BODANSKY, O. *Biochemistry of Disease*, 2nd ed. New York, 1952. Macmillan Co.
60. KING, E. J. Plasma alkaline phosphatase in disease. *Brit. M. Bull.*, 9: 160, 1953.
61. SNAPPER, I. *Bone Diseases in Medical Practice*. New York, 1957. Grune & Stratton.
62. THOMPSON, R. H. S. and KING, E. J. *Biochemical Disorders in Human Disease*. New York, 1957. Academic Press.
63. GUTMAN, A. B., GUTMAN, E. B. and KASABACH, H. H. Serum phosphatase activity in seventeen cases of osteoporosis circumscripta of the skull. *Proc. Soc. Exper. Biol. & Med.*, 33: 295, 1935.
64. KASABACH, H. H. and GUTMAN, A. B. Osteoporosis circumscripta of the skull and Paget's disease. Fifteen new cases and a review of the literature. *Am. J. Roentgenol.*, 37: 577, 1937.
65. BODANSKY, A. Phosphatase studies. II. Determination of serum phosphatase; factors influencing the accuracy of the determination. *J. Biol. Chem.*, 101: 93, 1933.
66. KING, E. J. and ARMSTRONG, A. R. A convenient method for determining serum and bile phosphatase activity. *Canad. M. A. J.*, 31: 376, 1934.
67. SHINOWARA, G. Y., JONES, L. M. and REINHART, H. L. The estimation of serum inorganic phosphate and "acid" and "alkaline" phosphatase activity. *J. Biol. Chem.*, 142: 921, 1942.
68. BESSEY, O. A., LOWRY, O. H. and BROCK, M. J. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem.*, 164: 321, 1946.
69. O'REILLY, T. J. and RACE, J. Osteitis deformans. *Quart. J. Med.*, 1: 471, 1932.
70. KAY, H. D., SIMPSON, S. L. and RIDDOCH, G. Osteitis deformans. *Arch. Int. Med.*, 53: 208, 1934.
71. DUVOIR, M., LAYANI, F. and PAUTRAT, J. Phosphatase sanguine et maladies osseuses. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 51: 1602, 1935.
72. JAFFE, H. L. and BODANSKY, A. Diagnostic significance of serum alkaline and acid phosphatase values in relation to bone disease. *Bull. New York Acad. Med.*, 19: 831, 1943.
73. ROSENKRANTZ, J. A., WOLF, J. and KAICHER, J. J. Paget's disease (osteitis deformans). Review of 111 cases. *Arch. Int. Med.*, 90: 610, 1952.
74. HIRSCH, W. *Die Ostitis deformans Paget*. Leipzig, 1953. Thieme.
75. WOODARD, H. Q. Long term studies of the blood chemistry in Paget's disease of bone. *Cancer*. (In press.)
76. GUTMAN, A. B. and GUTMAN, E. B. Paget's disease: Relative constancy of serum phosphatase levels over periods up to two years. *Proc. Soc. Exper. Biol. & Med.*, 33: 150, 1935.
77. ALBRIGHT, F. and HENNEMAN, P. H. The suppression of Paget's disease with ACTH and cortisone. *Tr. A. Am. Physicians*, 68: 238, 1955.
78. SULLIVAN, T. J., GUTMAN, E. B. and GUTMAN, A. B. Theory and application of the serum "acid" phosphatase determination in metastasizing prostatic carcinoma; early effects of castration. *J. Urol.*, 48: 426, 1942.
79. CHANGUS, G. W. Osteoblastic hyperplasia of bone. A histochemical appraisal of fibrous dysplasia of bone. *Cancer*, 10: 1157, 1957.
80. WOODARD, H. Q. Quantitative studies of betaglycerophosphatase activity in normal and neoplastic tissues. *Cancer*, 9: 352, 1956.
81. JEFFREE, G. M. Phosphatase activity in the limb bones of growing rabbits. *J. Bone & Joint Surg.*, 41B: 401, 1959.
82. ALBRIGHT, F., AUB, J. C. and BAUER, W. Hyperparathyroidism. *J. A. M. A.*, 102: 1276, 1934.
83. ALBRIGHT, F., SULKOWITCH, H. W. and BLOOMBERG, E. Further experience in the diagnosis of hyperparathyroidism, including a discussion of cases with a minimal degree of hyperparathyroidism. *Am. J. M. Sc.*, 193: 800, 1937.
84. KLENDSHOJ, N. C. and KOEPF, G. F. Serum phosphatase activity in hyperparathyroidism. *J. Clin. Endocrinol.*, 3: 351, 1943.
85. BODANSKY, A. and JAFFE, H. L. Plasma phosphatase in experimental hyperparathyroidism. *J. Biol. Chem.*, 92: xvi, 1931.
86. JAFFE, H. L. and BODANSKY, A. Experimental fibrous osteodystrophy (ostitis fibrosa) in hyperparathyroid dogs. *J. Exper. Med.*, 52: 629, 1930.
87. SMITH, J. and MAIZELS, M. The plasma phosphatase in rickets and scurvy. *Arch. Dis. Childhood*, 7: 149, 1932.
88. SMITH, J. Plasma phosphatase in rickets and other disorders of growth. *Arch. Dis. Childhood*, 8: 215, 1933.
89. BODANSKY, A. and JAFFE, H. L. Phosphatase studies. V. Serum phosphatase as a criterion of the severity and rate of healing of rickets. *Am. J. Dis. Child.*, 48: 1268, 1934.
90. STEARNS, G. and WARWEG, E. Studies of phosphorus of blood. III. The phosphorus partition in whole blood and in serum and the serum calcium and plasma phosphatase during healing of late rickets. *Am. J. Dis. Child.*, 49: 79, 1935.
91. BARNES, D. J. and CARPENTER, M. D. A comparative study in the diagnosis and treatment of rickets with observations of the normal and abnormal serum phosphatase. *J. Pediat.*, 10: 596, 1937.
92. MORRIS, N. The significance of plasma phosphatase in bone disease and jaundice. *Brit. M. J.*, 1: 91, 1937.
93. KLASMER, R. Serum phosphatase activity and clinical rickets in children in Jerusalem. *Am. J. Dis. Child.*, 67: 348, 1944.
94. GRAY, J. D. and CARTER, F. S. The early diagnosis of rickets. *Arch. Dis. Childhood*, 24: 189, 1949.
95. CORRELL, J. T. and WISE, E. C. Studies on the relative efficiency of vitamin D from several sources. II. Influence of vitamin D of different origins on the serum phosphatase of the chicken. *J. Biol. Chem.*, 126: 581, 1938.
96. MOTZOK, I. Studies on the plasma phosphatase of

- normal and rachitic chicks. iii. The assay of antirachitic preparations by a method based on the determination of plasma phosphatase activity. *Biochem. J.*, 47: 196, 1950.
97. MOTZOK, I. Studies on the plasma phosphatase of normal and rachitic chicks. ii. Relationship between plasma phosphatase and the phosphatases of bone, kidney, liver and intestinal mucosa. *Biochem. J.*, 47: 193, 1950.
 98. MOTZOK, I. Studies on alkaline phosphatases. i. Kinetics of plasma phosphatase of normal and rachitic chicks. *Biochem. J.*, 72: 169, 1959.
 99. CHU, H. I., LIU, S. H., YU, T. F., HSU, H. C., CHENG, T. Y. and CHAO, H. C. Calcium and phosphorus metabolism in osteomalacia. x. Further studies on vitamin D action: Early signs of depletion and effect of minimal doses. *J. Clin. Invest.*, 19: 349, 1940.
 100. SNAPPER, I. Medical Clinics on Bone Diseases. New York, 1949. Interscience.
 101. ALBRIGHT, F., BURNETT, C. H., PARSON, W., REIFENSTEIN, E. C., JR. and ROOS, A. Osteomalacia and late rickets. *Medicine*, 25: 399, 1946.
 102. PINES, K. L. and MUDGE, G. H. Renal tubular acidosis with osteomalacia. Report of three cases. *Am. J. Med.*, 11: 302, 1951.
 103. SNAPPER, I., SEELY, R., FALK, S. and FEDER, I. Osteomalacia in New York. *Ann. Int. Med.*, 41: 893, 1954.
 104. WINTERS, R. W., GRAHAM, J. B., WILLIAMS, T. F., MCFALLS, V. W. and BURNETT, C. H. A genetic study of familial hypophosphatemia and vitamin D-resistant rickets. *Tr. A. Am. Physicians*, 70: 234, 1957.
 105. McCUNE, D. J., MASON, H. H. and CLARKE, H. T. Intractable hypophosphatemic rickets with renal glycosuria and acidosis (the Fanconi syndrome). *Am. J. Dis. Child.*, 65: 81, 1943.
 106. STOWERS, J. M. and DENT, C. E. Studies on the mechanism of the Fanconi syndrome. *Quart. J. Med.*, 16: 275, 1947.
 107. SCHAJOWICZ, F. and CABRINI, R. H. Histochemical studies of bone in normal and pathological conditions with special reference to alkaline phosphatase, glycogen and mucopolysaccharides. *J. Bone & Joint Surg.*, 36B: 474, 1954.
 108. WOODARD, H. Q. Changes in blood chemistry associated with carcinoma metastatic to bone. *Cancer*, 6: 1219, 1953.
 109. NESBIT, R. M. and BAUM, W. C. Serum phosphatase determinations in diagnosis of prostatic cancer; a review of 1,150 cases. *J. A. M. A.*, 145: 1321, 1951.
 110. WOODARD, H. Q. and DEAN, A. L. The significance of phosphatase findings in carcinoma of the prostate. *J. Urol.*, 57: 158, 1947.
 111. WOODARD, H. Q. The clinical significance of serum acid phosphatase. *Am. J. Med.*, 27: 902, 1959.
 112. HUGGINS, C. and HODGES, C. V. Studies on prostatic cancer. i. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res.*, 1: 293, 1941.
 113. WRAY, S. The significance of the blood acid and alkaline phosphatase values in cancer of the prostate. *J. Clin. Path.*, 9: 341, 1956.
 114. WOODARD, H. Q., ESCHER, G. C. and FARROW, J. H. Changes in the blood chemistry of patients with disseminated carcinoma of the breast during endocrine therapy. *Cancer*, 7: 744, 1954.
 115. LINDSAY, M. K. Observations on fracture healing in rats. *J. Bone & Joint Surg.*, 16: 162, 1934.
 116. BOTTERELL, E. H. and KING, E. J. Phosphatase in fractures. *Lancet*, 1: 1267, 1935.
 117. WILKINS, W. E. and REGEN, E. M. Course of phosphatase activity in healing of fractured bone. *Proc. Soc. Exper. Biol. & Med.*, 32: 1373, 1935.
 118. MITCHELL, C. L. Serum phosphatase in fracture repair. *Ann. Surg.*, 104: 304, 1936.
 119. PEDEN, O. D. Plasma phosphatase and phosphorus during the healing of fractures in children. *Arch. Dis. Childhood*, 12: 87, 1937.
 120. WRIST, M. R. and JOHNSON, R. W., JR. Calcification and ossification. iv. The healing of fractures in man under clinical conditions. *J. Bone & Joint Surg.*, 25: 375, 1943.
 121. ROBINSON, J. N., GUTMAN, E. B. and GUTMAN, A. B. Clinical significance of increased serum "acid" phosphatase in patients with bone metastases secondary to prostatic carcinoma. *J. Urol.*, 42: 602, 1939.
 122. ENSIGN, D. C. Serum phosphatase in osteopetrosis. *J. Lab. & Clin. Med.*, 32: 1541, 1947.
 123. ROHOLM, K., GUTMAN, A. B. and GUTMAN, E. B. Serum phosphatase activity in generalized osteosclerosis due to chronic uric acid intoxication in man. *Proc. Soc. Exper. Biol. & Med.*, 37: 376, 1937.
 124. ALBRIGHT, F., BUTLER, A. M., HAMPTON, A. O. and SMITH, P. Syndrome characterized by osteitis fibrosa disseminata, areas of pigmentation and endocrine dysfunction, with precocious puberty in females; report of 5 cases. *J. Med.*, 216: 727, 1937.
 125. GUTMAN, A. B., SWENSON, P. C. and JONES, W. B. The differential diagnosis of hyperparathyroidism. *J. A. M. A.*, 103: 87, 1933.
 126. VERMEHREN, E. Variations in plasma phosphatase content in different ages. *Acta med. Scandinav.*, 100: 244, 1939.
 127. HARRISON, A. P., RODERICK, C., LESHER, M., KOUCHER, M., MOYER, E. Z., LAMECK, W. and BEACH, E. F. Nutritional status of children. vii. Blood serum phosphatase. *J. Am. Diet. A.*, 24: 503, 1948.
 128. CLARK, L. C. and BECK, E. Plasma "alkaline" phosphatase activity. i. Normative data for growing children. *J. Pediat.*, 36: 335, 1950.
 129. TALBOT, N. B., HOEFFEL, G., SHWACHMAN, H. and TUOHY, E. L. Serum phosphatase as an aid in the diagnosis of cretinism and juvenile hypothyroidism. *Am. J. Dis. Child.*, 62: 273, 1941.
 130. HILL, A. M. and WEBER, J. E. Serum phosphatase values in children showing retardation in osseous development and low metabolic rates. *J. Pediat.*, 22: 325, 1943.
 131. SHWACHMAN, H. Serum phosphatase in infantile scurvy. *J. Pediat.*, 19: 38, 1941.
 132. PERKINS, H. R. and ZILVA, S. S. The influence of scurvy and fall in weight in young guinea pigs on the alkaline phosphatase content of the serum and the zones of provisional calcification. *Biochem. J.*, 47: 306, 1950.

133. AUB, J. C., EVANS, R. D., GALAGHER, D. M. and TIBBETTS, D. M. Effects of treatment on radium and calcium metabolism in the human body. *Ann. Int. Med.*, 11: 1443, 1938.
134. RATHBUN, J. C. Hypophosphatasia, a new developmental anomaly. *Am. J. Dis. Child.*, 75: 822, 1948.
135. McCANCE, R. A., FAIRWEATHER, D. V. I., BARRETT, A. M. and MORRISON, A. B. Genetic, clinical, biochemical and pathological features of hypophosphatasia, based on the study of a family. *Quart. J. Med.*, 25: 523, 1956.
136. ENGFELDT, B. and ZETTERSTRÖM, R. Osteodysmorphismosis foetalis. A newly discovered characteristic skeletal disease showing low serum and tissue alkaline phosphatase activity (hypophosphatasia). In: Ciba Foundation Symposium on Bone Structure and Metabolism. Boston, 1956. Little, Brown & Co.
137. FRASER, D. Hypophosphatasia. *Am. J. Med.*, 22: 730, 1957.
138. CURRARINO, G., NEUHAUSER, E. B. D., REYERSBACH, G. C. and SOBEL, E. H. Hypophosphatasia. *Am. J. Roentgenol.*, 78: 392, 1957.
139. DICKSON, W. and HORROCKS, H. H. Hypophosphatasia. *J. Bone & Joint Surg.*, 40B: 64, 1958.
140. CUSWORTH, D. C. The isolation and identification of phosphoethanolamine from the urine of a case of hypophosphatasia. *Biochem. J.*, 68: 262, 1958.
141. HSIA, D. Y.-Y., KRAUS, M. and SAMUELS, J. Genetic studies on vitamin D resistant rickets (familial hypophosphatasemia). *Am. J. Human Genet.*, 11: 156, 1959.
142. BEISEL, W. R., BENJAMIN, N. and AUSTEN, K. F. Absence of leukocyte alkaline phosphatase activity in hypophosphatasia. *Blood*, 14: 975, 1959.
143. LICHTMAN, S. S. Diseases of the Liver, Gall Bladder and Bile Ducts, 3rd ed. Philadelphia, 1953. Lea & Febiger.
144. POPPER, H. and SCHAFFNER, F. Liver: Structure and Function. New York, 1957. Blakiston Division, McGraw-Hill Book Co.
145. GUTMAN, A. B. and HANGER, F. M., JR. Differential diagnosis of jaundice by combined serum phosphatase determination and cephalin flocculation test. *M. Clin. North America*, 25: 837, 1941.
146. GREENE, C. H. and BRUGER, M. The functional study of the liver and its clinical evaluation. *New York State J. Med.*, 43: 318, 1943.
147. MACLAGAN, N. F. Liver function tests in the diagnosis of jaundice. A review of 200 cases. *Brit. M. J.*, 2: 197, 1947.
148. SHAY, H. and SIPLET, H. Minimal yet adequate program of liver function studies in the differential diagnosis of jaundice. *Am. J. Med.*, 4: 215, 1948.
149. DUCCI, H. The flocculation tests in the differential diagnosis of jaundice. *Gastroenterology*, 15: 628, 1950.
150. HANGER, F. M. Working minimum of hepatic function tests for rationalizing the treatment of jaundice. *New York State J. Med.*, 50: 2188, 1950.
151. HANGER, F. M. The meaning of liver function tests. *Am. J. Med.*, 16: 565, 1954.
152. MACLAGAN, N. F. Liver function tests. In: Diseases of the Liver. Edited by Schiff, L. Philadelphia, 1956. J. B. Lippincott Co.
153. HILL, E. and ZIEVE, L. Discrimination between obstructive and hepatocellular jaundice by means of the commonly used serum tests. *Am. J. Clin. Path.*, 27: 6, 1957.
154. WROBLEWSKI, F. The significance of alterations in serum enzymes in the differential diagnosis of jaundice. *Arch. Int. Med.*, 100: 635, 1957.
155. WROBLEWSKI, F. Increasing clinical significance of alterations in enzymes of body fluids. *Ann. Int. Med.*, 50: 62, 1959.
156. DIXON, T. F. and PURDOM, M. Serum 5-nucleotidase. *J. Clin. Path.*, 7: 341, 1954.
157. YOUNG, I. I. Serum 5-nucleotidase: characterization and evaluation in disease states. *Ann. New York Acad. Sci.*, 75: 357, 1958.
158. HANGER, F. M. Serological differentiation of obstructive from hepatogenous jaundice by flocculation of cephalin-cholesterol emulsions. *J. Clin. Invest.*, 18: 261, 1938.
159. GIORDANO, A. S., WILHELM, A. and PRESTRUD, M. C. The serum phosphatase in the differential diagnosis of obstructive jaundice. *Am. J. Clin. Path.*, 9: 226, 1939.
160. SCHWIMMER, D., KLOTZ, S. D., DREKTER, I. J. and MCGAVACK, T. H. A fasting blood sample procedure in the differential diagnosis and management of hepatic disease. *Am. J. Digest. Dis.*, 12: 1, 1945.
161. VAN DER MEER, P. The phosphatase content of the serum in jaundice. *Acta med. Scandinav.*, 126: 265, 1946.
162. ARIEL, I. M. and SHAHON, D. B. Hepatic dysfunction in candidates for abdominal surgery, especially in patients with cancer. *Cancer*, 3: 608, 1950.
163. ULEVITCH, H., GALL, E. A., HOXWORTH, P. I., SCHIFF, L. and GRALLER, D. L. The importance of serial determinations of serum alkaline phosphatase in incomplete biliary tract obstruction. *J. Lab. & Clin. Med.*, 38: 693, 1951.
164. BREM, T. H. Recognition of biliary tract obstruction without jaundice. *J. A. M. A.*, 159: 1624, 1955.
165. CULVER, P. J., McDERMOTT, W. V. and JONES, C. M. Diagnostic value of selective interference with certain excretory processes of the liver. *Gastroenterology*, 33: 163, 1957.
166. GIBBONS, T. B. Hyperphosphatasemia in patients without jaundice with hepatobiliary disease. *J. A. M. A.*, 164: 22, 1957.
167. HOFFBAUER, F. W. Clinical aspects of jaundice resulting from intrahepatic obstruction. *J. A. M. A.*, 169: 1453, 1959.
168. POPPER, H. and SCHAFFNER, F. Pathology of jaundice resulting from intrahepatic cholestasis. *J. A. M. A.*, 169: 1447, 1959.
169. BULLARD, R. W., JR. Alkaline phosphatase and metastatic liver disease. *Surgery*, 19: 379, 1946.
170. MENDELSON, M. L. and BODANSKY, O. The value of liver-function tests in the diagnosis of intrahepatic metastases in the nonicteric patient. *Cancer*, 5: 1, 1952.
171. THOMAS, C. J. and ZIMMERMAN, H. J. The pattern of abnormality of liver function in metastatic carcinoma. *J. Lab. & Clin. Med.*, 39: 882, 1952.
172. EUSTERMAN, G. B. Significance of liver function tests in diagnosis of intrahepatic metastases in non-icteric cancer patient, with special reference

- to alkaline phosphatase test. *Bull. Mt. Sinai Hosp., Detroit*, 1: 7, 1953.
173. SHAY, H. and SIPLET, H. The value of serum alkaline phosphatase determination and bromsulphalein test in the diagnosis of metastatic cancer of the liver. *J. Lab. & Clin. Med.*, 43: 741, 1954.
 174. SIMON, R. L. Hepatic tests in metastatic carcinoma of the liver; a review of seventy-three cases proven at necropsy. *Am. J. M. Sc.*, 228: 312, 1954.
 175. LÜHRS, W., GUMMEL, H. and KINDERMANN, I. Alkalische Serumphosphatase und Lebermetastasierung. *Ztschr. ges. inn. med.*, 10: 713, 1955.
 176. BODANSKY, O. Blood biochemical alterations in neoplastic disease. *M. Clin. North America*, p. 611, 1956.
 177. JONGH, M. L., HELLENDORF, G. B. A. and GERBRANDY, J. Alkaline serum phosphatase activity in cases of metastasized tumours. *Nederl. tijdschr. v. geneesk.*, 101: 859, 1957.
 178. FITZSIMMONS, E. J., SOMMERS, H. M. and LAIPPLY, T. C. The value of simultaneous urine bilirubin and serum alkaline phosphatase analyses in the diagnosis of intrahepatic metastases of carcinoma. *J. Lab. & Clin. Med.*, 54: 192, 1959.
 179. SHARNOFF, J. G., LISA, J. R. and RIEDEL, P. A. Serum phosphatase activity in disease of the liver. Correlation of the serum enzyme activity and the hepatic histologic changes. *Arch. Path.*, 33: 460, 1942.
 180. HARRELL, G. T. and FISHER, S. Blood chemical changes in Boeck's sarcoid with particular reference to protein, calcium and phosphatase values. *J. Clin. Invest.*, 18: 687, 1939.
 181. KLATSKIN, G. and YESNER, R. Hepatic manifestations of sarcoidosis and other granulomatous diseases; a study based on histological examination of tissue obtained by needle biopsy of the liver. *Yale J. Biol. & Med.*, 23: 207, 1950.
 182. RILEY, E. A. Boeck's sarcoid. A review based upon a clinical study of fifty-two cases. *Am. Rev. Tuberc.*, 62: 231, 1950.
 183. SHAY, H., BERK, J. E., SONES, M., AEGERTER, E. E., WESTON, J. K. and ADAMS, A. B. The liver in sarcoidosis. *Gastroenterology*, 19: 441, 1951.
 184. ROSS, P. H. and WEINBERG, B. J. Chronic regurgitation jaundice as the presenting sign in sarcoidosis. *Arch. Int. Med.*, 87: 269, 1951.
 185. CLEVE, E. A., GIBSON, J. R. and WEBB, W. M. Atypical tuberculosis of the liver with jaundice. *Ann. Int. Med.*, 41: 251, 1954.
 186. KORN, R. J., KELLOW, W. F., HELLER, P., CHOMET, B. and ZIMMERMAN, H. J. Hepatic involvement in extrapulmonary tuberculosis; histologic and functional characteristics. *Am. J. Med.*, 27: 60, 1959.
 187. LEVITAN, R., DIAMOND, H. D. and CRAVER, L. F. Jaundice in Hodgkin's disease. *Am. J. Med.* (In press.)
 188. SPAIN, D. M. and RILEY, R. L. Jaundice in amyloidosis of the liver. *Am. J. Clin. Path.*, 14: 284, 1944.
 189. KLATSKIN, G. Toxic hepatitis. In: *Diseases of the Liver*, p. 328. Edited by Schiff, L. Philadelphia, 1956. J. B. Lippincott.
 190. POPPER, H. and SCHAFFNER, F. Drug-induced hepatic injury. *Ann. Int. Med.* (In press.)
 191. GUTMAN, A. B. Drug reactions characterized by cholestasis associated with intrahepatic biliary tract obstruction. *Am. J. Med.*, 23: 841, 1957.
 192. HOLLISTER, L. E. Allergy to chlorpromazine manifested by jaundice. *Am. J. Med.*, 23: 870, 1957.
 193. WERTHER, J. L. and KORELITZ, B. I. Chlorpromazine jaundice. Analysis of twenty-two cases. *Am. J. Med.*, 22: 351, 1957.
 194. SHAY, H. and SIPLET, H. Study of chlorpromazine jaundice, its mechanism and prevention, with special reference to serum alkaline phosphatase and glutamic oxalacetic transaminase. *Gastroenterology*, 32: 571, 1957.
 195. ZELMAN, S. Liver cell necrosis in chlorpromazine jaundice (allergic cholangiolitis). A serial study of twenty-six needle biopsy specimens in nine patients. *Am. J. Med.*, 27: 708, 1959.
 196. EPPINGER, H. Die Leberkrankheiten. Allgemeine und spezielle Pathologie und Therapie der Leber. Vienna, 1937. Julius Springer.
 197. RÖSSLE, R. Entzündung der Leber. In: *Handbuch der speziellen pathologischen Anatomie und Histologie*, vol. 5, part #1. Edited by Henke, F. and Lubarsch, O. Berlin, 1930. Julius Springer.
 198. WATSON, C. J. and HOFFBAUER, W. The problem of prolonged hepatitis with particular reference to the cholangiolitic type and to the development of cholangiolitic cirrhosis of the liver. *Ann. Int. Med.*, 25: 195, 1946.
 199. CHANUTIN, A. and LUDEWIG, S. Blood lipid studies of cases of xanthomatosis associated with hepatic damage. *J. Lab. & Clin. Med.*, 12: 303, 1937.
 200. STOLZER, B. L., MILLER, G., WHITE, W. A. and ZUCKERBROD, M. Postarsenical obstructive jaundice complicated by xanthomatosis and diabetes mellitus. *Am. J. Med.*, 9: 124, 1950.
 201. MYERS, J. D., OLSON, R. E., LEWIS, J. H. and MORAN, T. J. Xanthomatous biliary cirrhosis following chlorpromazine, with observations indicating overproduction of cholesterol, hyperprothrombinemia, and the development of portal hypertension. *Tr. A. Am. Physicians*, 70: 243, 1957.
 202. AHRENS, E. H., JR., PAYNE, M. A., KUNKEL, H. G., EISENMENGER, W. J. and BLONDHEIM, S. H. Primary biliary cirrhosis. *Medicine*, 29: 299, 1950.
 203. RICKETTS, W. E. and WISSLER, R. W. Cholangiolitic biliary cirrhosis (primary biliary cirrhosis). *Ann. Int. Med.*, 36: 1241, 1952.
 204. CAMERON, R. Some problems of biliary cirrhosis. *Brit. M. J.*, 1: 535, 1958.
 205. MACLAGEN, N. F. Laboratory tests in the diagnosis of liver disease. A report on three procedures. *Brit. M. J.*, 2: 363, 1944.
 206. SHERLOCK, S. Biochemical investigations in liver disease; some correlations with hepatic histology. *J. Path. & Bact.*, 58: 523, 1946.
 207. HOAGLAND, C. L. and SHANK, R. E. Infectious hepatitis: A review of 200 cases. *J. A. M. A.*, 130: 615, 1946.
 208. MACFEE, W. F. Painless jaundice. *J. A. M. A.*, 141: 171, 1949.
 209. DAHMS, H. Zur klinischen Brauchbarkeit der

- alkalischen Phosphataseprobe. *Klin. Wchnschr.*, 30: 830, 1952.
210. SIMMER, H. Die sogenannte alkalische Serumphosphatase. Zur Differentialdiagnose ikterischer Erkrankungen der Leber und der Gallenwege. *Deutsche med. Wchnschr.*, 81: 2108, 1956.
 211. EGGHART, F. Die alkalische Serumphosphatase bei Erkrankungen der Leber und des Gallensystems. *Wien. Ztschr. inn. Med.*, 38: 518, 1957.
 212. BODANSKY, A. Non-osseous origins of serum phosphatase: The liver. *Enzymologia*, 3: 258, 1937.
 213. WINKELMAN, L. and SCHIFFMANN, A. Clinical significance of variations in serum phosphatase in hepatic and biliary disorders. *Arch. Int. Med.*, 64: 348, 1939.
 214. BURKE, J. O. Serum alkaline phosphatase in liver disease; a concept of its significance. *Gastroenterology*, 16: 660, 1950.
 215. GALL, E. A. and BRAUNSTEIN, H. Hepatitis with manifestations simulating bile duct obstruction (so-called "cholangiolitic hepatitis"). *Am. J. Clin. Path.*, 25: 1113, 1955.
 216. JOHNSON, H. C., JR. and DOENGES, J. P. Intrahepatic obstructive jaundice (primary cholestasis), a clinico-pathologic syndrome of varied etiology: a review with observations of the use of corticotropin as a diagnostic tool. *Ann. Int. Med.*, 44: 589, 1956.
 217. OVERHOLT, E. L. and HARDIN, E. B. Cholangiolitic hepatitis. *Arch. Int. Med.*, 103: 859, 1959.
 218. COHN, C. and LIDMAN, B. I. Hepatitis without jaundice in infectious mononucleosis. *J. Clin. Invest.*, 25: 145, 1946.
 219. GALL, E. A. Serum phosphatase and other tests of liver function in infectious mononucleosis. *Am. J. Clin. Path.*, 17: 529, 1947.
 220. PETERSON, R. E. Hepatic dysfunction in infectious mononucleosis; with review of the literature. *J. Lab. & Clin. Med.*, 33: 1258, 1948.
 221. JORDAN, W. S. and ALBRIGHT, R. W. Liver function tests in infectious mononucleosis. *J. Lab. & Clin. Med.*, 35: 688, 1950.
 222. SCHULTZ, A. L. and HALL, W. H. Clinical observations in 100 cases of infectious mononucleosis and the results of treatment with penicillin and aureomycin. *Ann. Int. Med.*, 36: 1498, 1952.
 223. WORMER, D. C., MARTIN, W. J. and WOLLAEGER, E. E. Infectious mononucleosis with jaundice and abdominal pain as presenting complaints: Report of a case. *Ann. Int. Med.*, 45: 718, 1956.
 224. BARONDESS, J. A. and ERLE, H. Serum alkaline phosphatase activity in hepatitis of infectious mononucleosis. *Am. J. Med.* (In press.)
 225. MACDONALD, R. A. and MALLORY, G. K. The natural history of postnecrotic cirrhosis. A study of 221 autopsy cases. *Am. J. Med.*, 24: 334, 1958.
 226. SPATT, S. D. and GRAYZEL, D. M. Primary carcinoma of the liver. *Am. J. Med.*, 5: 570, 1948.
 227. GALLUZZI, N. J., WEINGARTEN, W., REGAN, F. D. and DOERNER, A. A. Evaluation of hepatic tests and clinical findings in primary hepatic cancer. *J. A. M. A.*, 152: 15, 1953.
 228. Regulation of Cell Metabolism. Ciba Foundation Symposium. Edited by Wolstenholme, G. E. W. and O'Connor, C. M. Boston, 1959. Little, Brown & Co.
 229. GOMORI, G. Microscopic Microchemistry. Principle and Practice. Chicago, 1952. University of Chicago Press.
 230. GUTMAN, A. B. and JONES, B. Inhibition by cyanide of serum alkaline phosphatase in normal man, obstructive jaundice and skeletal disorders. *Proc. Soc. Exper. Biol. & Med.*, 71: 572, 1949.
 231. STETTEN, DEW., JR. Thermodynamic, kinetic and biologic stability. *Am. J. Med.*, 13: 251, 1952.
 232. BODANSKY, A. and JAFFE, H. L. Effects of diet and fasting on plasma phosphatase. *Proc. Soc. Exper. Biol. & Med.*, 29: 199, 1931.
 233. BODANSKY, A. Phosphatase studies. VII. Inorganic phosphorus and phosphatase of the serum in new born puppies. *J. Biol. Chem.*, 104: 717, 1934.
 234. GUTMAN, A. B., HOGG, B. M. and OLSON, K. B. Increased serum phosphatase activity without hyperbilirubinemia after ligation of hepatic ducts in dogs. *Proc. Soc. Exper. Biol. & Med.*, 44: 613, 1940.
 235. SPELLBERG, M. A. Liver function tests: Their clinical significance with special emphasis on elevation of alkaline phosphatase and cholesterol. *Am. J. Gastroenterol.*, 28: 234, 1957.
 236. CANTAROW, A., STEWART, H. L. and MCCOOL, S. G. Serum phosphatase in cats with total bile stasis. *Proc. Soc. Exper. Biol. & Med.*, 35: 87, 1936.
 237. FLOOD, C. A., GUTMAN, E. B. and GUTMAN, A. B. Serum and urine phosphatase activity in the cat after ligation of the common bile duct. *Am. J. Physiol.*, 120: 696, 1937.
 238. THANNHAUSER, S. J., REICHEL, M., GRATAN, J. F. and MADDOCK, J. J. Studies on serum phosphatase activity. II. The effect of experimental total biliary obstruction on the serum phosphatase activation in dogs and cats. *J. Biol. Chem.*, 121: 709, 1937.
 239. DALGAARD, J. B. Phosphatase in cats with obstructive jaundice. *Acta physiol. Scandinav.*, 15: 290, 1948.
 240. KRITZLER, R. A. and BEAUBIEN, J. Microchemical variation of alkaline phosphatase activity of liver in obstructive and hepatocellular jaundice. *Am. J. Path.*, 25: 1079, 1949.
 241. WILMER, H. A. Renal phosphatase. The correlation between the functional activity of the renal tubule and its phosphatase content. *Arch. Path.*, 37: 227, 1944.
 242. WEIL, L. and RUSSELL, M. A. Studies on plasma phosphatase activity in relation to fat metabolism in rats. *J. Biol. Chem.*, 136: 9, 1940.
 243. GOULD, B. S. Studies on the source of serum phosphatase. The nature of the increased serum phosphatase in rats after fat feeding. *Arch. Biochem.*, 4: 175, 1944.
 244. FLOCK, E. V. and BOLLMAN, J. D. Alkaline phosphatase in the intestinal lymph of the rat. *J. Biol. Chem.*, 175: 439, 1948.
 245. FLOCK, E. V. and BOLLMAN, J. L. The influence of bile on the alkaline phosphatase activity of intestinal lymph. *J. Biol. Chem.*, 184: 523, 1950.
 246. MADSEN, N. B. and TUBA, J. On the source of the alkaline phosphatase in rat serum. *J. Biol. Chem.*, 195: 741, 1952.
 247. JACKSON, S. H. The effect of food ingestion on in-

- testinal and serum alkaline phosphatase in rats. *J. Biol. Chem.*, 198: 553, 1952.
248. MADDOCK, S., THANNHAUSER, S. J., REICHEL, M. and GRATTAN, J. A new conception of serum phosphatase. Review of experimental work. *New England J. Med.*, 218, 166, 1938.
 249. CANTAROW, A. The influence of icteric sera with high phosphatase activity on the phosphatase activity of normal sera. *Am. J. Clin. Path.*, 10: 858, 1940.
 250. BODANSKY, O. Diagnostic applications of enzymes in medicine. General enzymological aspects. *Am. J. Med.*, 27: 861, 1959.
 251. BODANSKY, O. The accelerant effect of α -amino acids on the activity of bone phosphatase. *J. Biol. Chem.*, 114: 273, 1936.
 252. BODANSKY, O. Are the phosphatases of bone, kidney, intestine and serum identical? *J. Biol. Chem.*, 118: 341, 1937.
 253. HENNEMAN, P. H., ROURKE, G. M. and JACKSON, W. P. V. Depression of serum alkaline phosphatase activity by human serum albumin. *J. Biol. Chem.*, 213: 19, 1955.
 254. KING, E. J. and DELORY, G. E. Phosphatase activity. *Chem. & Ind.*, 57: 85, 1938.
 255. THANNHAUSER, S. J., REICHEL, M., GRATTAN, J. F. and MADDOCK, S. J. Studies on serum phosphatase activity. VI. The influence of sera with high phosphatase activity on normal sera. *J. Biol. Chem.*, 124: 631, 1938.
 256. ALBERS, D. Über sog. Aktivatoren der alkalischen Phosphatasen. *Ztschr. f. physiol. Chem.*, 266: 1, 1940.
 257. WILLIAMS, H. L. and WATSON, E. M. The hyperphosphatasemia of Paget's disease. *J. Lab. & Clin. Med.*, 26: 1333, 1941.
 258. DELORY, C. E. and KING, E. J. The "activation" of phosphatase. *Biochem. J.*, 38: 50, 1944.
 259. DALGAARD, J. B. Serum and bile phosphatase in biliary fistula dogs. *Acta physiol. Scandinav.*, 16: 293, 1949.
 260. WACHSTEIN, M. and ZAK, F. G. Histochemical distribution of alkaline phosphatase in dog liver after experimental biliary obstruction. *Proc. Soc. Exper. Biol. & Med.*, 62: 73, 1946.
 261. WACHSTEIN, M. and ZAK, F. G. Distribution of alkaline phosphatase in the human liver. A study of postmortem material. *Arch. Path.*, 42: 501, 1946.
 262. SHERLOCK, S. and WALSHE, V. Hepatic alkaline phosphatase; histological and microchemical studies on liver tissue in normal subjects and in liver and bone disease. *J. Path. & Bact.*, 59: 615, 1947.
 263. HARD, W. L. and HAWKINS, R. K. The role of the bile capillaries in the secretion of phosphatase by the rabbit liver. *Anat. Rec.*, 106: 395, 1950.
 264. HANZON, V. Liver cell secretion under normal and pathologic conditions studied by fluorescence microscopy on living rats. *Acta physiol. Scandinav.* (Supp. 101), 28: 1-268, 1952.
 265. HANZON, V. Dye secretion and dye uptake by the liver. In: *Liver Function*. Edited by Brauer, R. W. Washington, D. C., 1958. American Institute of Biological Sciences.
 266. WROBLEWSKI, F. The clinical significance of transaminase activities of serum. *Am. J. Med.*, 27: 911, 1959.
 267. KOIDE, H. and ODA, T. Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. *Clin. Chim. Acta*, 4: 554, 1959.
 268. SHERLOCK, S. *Diseases of the Liver and Biliary System*. Oxford, 1955. Blackwell Scientific Publications.
 269. TAFT, E. B. and SCOTT, J. F. Studies in histochemistry. II. Quantitative histochemical estimation of activities of alkaline and acid phosphomonoesterases. *J. Histochem. & Cytochem.*, 6: 340, 1958.
 270. GREENSTEIN, J. P. Distribution of acid and alkaline phosphatase in tumors, normal tissues and the tissues of tumor-bearing rats and mice. *J. Nat. Cancer Inst.*, 2: 511, 1942.
 271. *Enzymes: Units of Biological Structure and Function*. New York, 1956. Edited by Gaebler, O. H. Academic Press.
 272. PAVEL, I. *Les Ictères*, 3rd ed. Paris, 1949. Masson.
 273. EDLUND, Y. and HANZON, V. Demonstration of the close relationship between bile capillaries and sinusoid walls. *Acta anat.*, 17: 105, 1953.
 274. ROULLER, C. Les canalicules biliaires. Étude au microscope électronique. *Acta anat.*, 26: 94, 1956.
 275. BRAUER, R. W. Mechanisms of bile secretion. *J. A. M. A.*, 169: 1462, 1959.
 276. MANN, F. C. and BOLLMAN, J. L. Jaundice: Review of some experimental investigations. *J. A. M. A.*, 104: 371, 1935.
 277. ARMSTRONG, A. R. and BANTING, F. G. The site of formation of the phosphatase of serum. *Canad. M. A. J.*, 33: 243, 1935.
 278. MADDOCK, S., TRIMBLE, H. C., JENSEN, D. and APPELBY, W. The effect of hepatectomy, and abdominal evisceration with and without hepatectomy on the serum phosphatase of the dog. *Am. J. Path.*, 17: 456, 1941.
 279. DALGAARD, J. B. Serum phosphatase after hepatectomy in dogs. *Acta physiol. Scandinav.*, 16: 308, 1949.
 280. FREEMAN, S. Comparison of effects of hepatectomy and of common bile duct obstruction on serum phosphatase of adult dogs. *Am. J. Physiol.*, 164: 792, 1951.
 281. FLOCK, E. V., BLOCK, M. A., BOLLMAN, J. L. and MANN, F. C. Alkaline phosphatase and amylase of plasma after hepatectomy. *Am. J. Physiol.*, 170: 467, 1952.
 282. DALGAARD, J. B. Phosphatase in dogs following bile obstruction and removal of the small intestine. *Acta physiol. Scandinav.*, 16: 287, 1949.
 283. REINHOLD, J. G. Chemical evaluation of the functions of the liver. *Clin. Chem.*, 1: 351, 1955.
 284. FREEMAN, S., CHEN, Y. P. and IVY, A. C. On the cause of the elevation of serum phosphatase in jaundice. *J. Biol. Chem.*, 124: 79, 1938.
 285. OPPENHEIMER, M. J. and FLOCK, E. V. Alkaline phosphatase levels in plasma and liver following partial hepatectomy. *Am. J. Physiol.*, 149: 418, 1947.
 286. NORBERG, B. On proteins in regenerating rat liver. I. Phosphomonoesterases. *Acta physiol. Scandinav.*, 19: 246, 1949.
 287. GOODLAD, G. A. J., MILLS, G. T. and SMITH, E. E. B.

- Studies on rat-liver phosphatase. *Biochem. J.*, 49: vi, 1951.
288. SCHIFFMANN, A. and WINKELMAN, L. Influence of the liver on serum phosphatase. *Arch. Int. Med.*, 63: 919, 1939.
 289. CINELLI, A. P. Influencia de la bilis administrada por boca sobre el aumento de fosfatasa sanguínea, provocado por obstrucción biliar. *Rev. soc. argent. biol.*, 18: 53, 1942.
 290. GAD, I. Investigations of the phosphatase activity in serum and organs after ligation of the common bile duct in dogs. *Acta physiol. Scandinav.*, 11: 151, 1946.
 291. CARR, J. L. and FOOTE, F. S. Alkaline and acid phosphatase levels in the serum of dogs after ligation of the common bile duct. *Arch. Surg.*, 49: 44, 1944.
 292. THANNHAUSER, S. J., REICHEL, M., GRATTAN, J. F. and MADDOCK, S. Studies on serum phosphatase activity. III. The effect of complete biliary fistula on phosphatase activity in serum and bile. *J. Biol. Chem.*, 121: 715, 1937.
 293. DRILL, V. A., ANNEGERS, J. A., SNAPP, E. F. and IVY, A. C. Effect of biliary fistula on bromsulphalein retention, serum phosphatase and bile phosphatase. *J. Clin. Invest.*, 24: 97, 1945.
 294. FREEMAN, S. and CHEN, Y. P. The effect of jaundiced blood or serum upon the serum phosphatase, inorganic phosphorus, and sugar of normal dogs. *Am. J. Physiol.*, 119: 311, 1937.
 295. FREEMAN, S. and YENG, P. C. The effect of jaundiced blood upon normal dogs with special reference to the serum phosphatase. *J. Biol. Chem.*, 123: 239, 1938.
 296. CANTAROW, A. and MILLER, L. L. Nonexcretion of jaundice-serum alkaline phosphatase in bile of normal dogs. *Am. J. Physiol.*, 153: 444, 1948.
 297. WANG, C. C. and GROSSMAN, M. I. Nonexcretion of serum alkaline phosphatase by the liver and the pancreas of normal dogs. *Am. J. Physiol.*, 156: 256, 1949.
 298. CANTAROW, A. The effect of jaundiced serum upon the phosphatase activity. *Am. J. Physiol.*, 126: 457, 1939.
 299. MADDOCK, S., SCHMIDT, G. and THANNHAUSER, S. J. The effect of the intravenous injection of alkaline intestinal phosphatase on dogs following hepatectomy and total abdominal visceration with and without hepatectomy. *Fed. Proc.*, 1: 181, 1942.
 300. LEVEEN, H. H., TALBOT, L. J., RESTUCCIA, M. and BARBERIO, J. R. Metabolism and excretion of alkaline phosphatase: Relation to liver function and determination of maximum secreting rates of the liver. *J. Lab. & Clin. Med.*, 36: 192, 1950.
 301. HARTMAN, F. W. and SCHELLING, V. Serum phosphatase in experimental insufficiency of the liver. *Arch. Path.*, 18: 594, 1934.
 302. ARMSTRONG, A. R. and KING, E. J. Serum phosphatase in toxic and haemolytic jaundice. *Canad. M. A. J.*, 32: 379, 1935.
 303. ROCHE, J. and THOAI, N-V. Phosphatase alcaline. *Advances Enzymol.*, 10: 83, 1950.
 304. BELFANTI, S., CONTARDI, A. and ERCOLI, A. Researches on the phosphatases. III. On the mechanism of the inactivating action of sodium oxalate and of phosphates on the "alkaline" phosphatases of animal tissue. *Biochem. J.*, 29: 1491, 1935.
 305. CLOETENS, R. Identification de deux phosphatases "alcalines" dans les organes animaux. *Enzymologia*, 6: 46, 1939.
 306. BODANSKY, O. The influence of magnesium and cobalt on the inhibition of phosphatases of bone, intestine and osteogenic sarcoma by amino acids. *J. Biol. Chem.*, 179: 81, 1949.
 307. DRILL, V. A., ANNEGERS, J. H. and IVY, A. C. Effect of cyanide, fluoride and magnesium on serum phosphatase activity during hepatic damage. *J. Biol. Chem.*, 152: 339, 1944.
 308. DRILL, V. A. and RIGGS, D. S. Inhibition of alkaline serum phosphatase activity during liver disease. *J. Biol. Chem.*, 162: 21, 1946.
 309. BOMAN, H. G. and WESTLUND, L. E. Protein chromatography on an anion-exchange resin. *Arch. Biochem.*, 64: 217, 1956.
 310. FAHEY, J. L., MCCOY, P. F. and GOULIAN, M. Chromatography of serum proteins in normal and pathologic sera: The distribution of protein-bound carbohydrate and cholesterol, siderophilin, thyroxin-binding protein, B₁₂-binding protein, alkaline and acid phosphatases, radio-iodinated albumin and myeloma proteins. *J. Clin. Invest.*, 37: 272, 1958.
 311. DELCOURT, A. and DELCOURT, R. Séparation et localisation des enzymes dans les liquides organiques par électrophorèse sur papier. *Compt. rend. Soc. de Biol.*, 147: 1104, 1953.
 312. SCHLAMOWITZ, M. Immunochemical studies on alkaline phosphatase. *Ann. New York Acad. Sc.*, 75: 373, 1958.
 313. SCHLAMOWITZ, M. and BODANSKY, O. Tissue sources of human serum alkaline phosphatase, as determined by immunochemical procedures. *J. Biol. Chem.*, 234: 1433, 1959.
 314. Discussion in: Regulation of Cell Metabolism. Ciba Foundation Symposium, p. 332. Edited by Wolstenholme, G. E. W. and O'Connor, C. M. Boston, 1959. Little, Brown & Co.
 315. GROSSER, P. and HUSLER, J. Über das Vorkommen einer Glycerophosphatase in tierischen Organen. *Biochem. Ztschr.*, 39: 1, 1912.
 316. DAVIDSON, J. N. Nucleoproteins, nucleic acids, and derived substances. *Ann. Rev. Biochem.*, 18: 155, 1949.
 317. BÉLANGER, L. F. Detection of phosphatase activity in polarized light following glycerophosphate incubation. *Proc. Soc. Exper. Biol. & Med.*, 77: 266, 1951.
 318. JOHNSON, F. R. and McMINN, R. M. H. The association of alkaline phosphatase with fibrogenesis. *J. Anat.*, 92: 544, 1958.
 319. VORBRÖDT, A. Histochemically demonstrable phosphatases and protein syntheses. *Exper. Cell Res.*, 15: 1, 1958.
 320. KROON, D. B. Phosphatase and the formation of protein-carbohydrate complexes. *Acta anat.*, 15: 317, 1952.
 321. RACKER, E. Mechanism of action and properties of pyridine nucleotide-linked enzymes. *Physiol. Rev.*, 35: 1, 1955.
 322. GUTMAN, A. B. and YÜ, T. F. A concept of the role of enzymes in endochondral calcification. In:

- Transactions of the Second Macy Conference in Metabolic Interrelations, p. 167, 1950.
323. GUTMAN, A. B. Current theories of bone salt formation, with special reference to enzyme mechanisms in endochondral calcification. *Bull. Hosp. Joint Dis.*, 12: 74, 1951.
 324. PICARD, J. Relations entre la Mineralisation du Cartilage Ossifiable et son Metabolisme chez l'Embryon de Mouton et le Rat. Paris, 1955. S. Vaillant.
 325. McLEAN, F. C. and URIST, M. R. Bone, an Introduction to the Physiology of Skeletal Tissue. Chicago, 1955. University of Chicago Press.
 326. BOURNE, G. H. Phosphatase and bone. In: The Biochemistry and Physiology of Bone, p. 251. Edited by Bourne, G. H. New York, 1956. Academic Press.
 327. DIXON, T. F. and PERKINS, H. R. The chemistry of calcification. In: The Biochemistry and Physiology of Bone, p. 287. Edited by Bourne, G. H. New York, 1956. Academic Press.
 328. Bone Structure and Metabolism. Ciba Foundation Symposium. Edited by Wolstenholme, G. E. W. and O'Connor, C. M. Boston, 1956. Little, Brown & Co.
 329. EEG-LARSEN, N. An experimental study on growth and glycolysis in the epiphyseal cartilage of rats. *Acta physiol. Scandinav.* (suppl. 128), 38: 1, 1956.
 330. HENRICHSEN, E. Alkaline Phosphatase and Calcification. Histochemical Investigations on the Relationship between Alkaline Phosphatase and Calcification. Copenhagen, 1958. E. Munksgaard.
 331. GOMORI, G. Calcification and phosphatase. *Am. J. Path.*, 19: 197, 1943.
 332. LORCH, I. J. Alkaline phosphatase and the mechanism of ossification. *J. Bone & Joint Surg.*, 31B: 94, 1949.
 333. HENRICHSEN, E. Alkaline phosphatase in osteoblasts and fibroblasts cultivated in vitro. *Exper. Cell Res.*, 11: 115, 1956.
 334. GUTMAN, A. B. and GUTMAN, E. B. A phosphorylase in calcifying cartilage. *Proc. Soc. Exper. Biol. & Med.*, 48: 687, 1941.
 335. GUTMAN, A. B., WARRICK, F. B. and GUTMAN, E. B. Phosphorylative glycogenolysis and calcification in cartilage. *Science*, 95: 461, 1942.
 336. GUTMAN, A. B. and YÜ, T. F. Further studies of the relation between glycogenolysis and calcification in cartilage. In: Transactions of the First Macy Conference on Metabolic Interrelations, p. 11, 1949.
 337. MARKS, P. A. and SHORR, E. Factors which regulate the deposition of calcium and strontium in rachitic cartilage in vitro. In: Transactions Second Macy Conference on Metabolic Interrelations, p. 191, 1950.
 338. ROCHE, J. Sur la biochimie générale des phosphatases. *Helv. chim. acta*, 29: 1253, 1946.
 339. SIFFERT, R. S. The role of alkaline phosphatase in osteogenesis. *J. Exper. Med.*, 93: 415, 1951.
 340. NEUMAN, W. F., DiSTEFANO, V. and MULRYAN, B. J. Role of phosphatase. *Fed. Proc.*, 10: 228, 1951.
 341. NEUMAN, W. F., DiSTEFANO, V. and MULRYAN, B. J. The surface chemistry of bone. III. Observations on the role of phosphatase. *J. Biol. Chem.*, 193: 227, 1951.
 342. NEUMAN, W. F. and NEUMAN, M. W. The Clinical Dynamics of Bone Mineral. Chicago, 1958. University of Chicago Press.
 343. DiSTEFANO, V., NEUMAN, W. F. and ROUSER, G. Isolation of a phosphate ester from calcifiable cartilage. *Arch. Biochem. & Biophys.*, 47: 219, 1953.
 344. STRATES, B. and NEUMAN, W. F. On the mechanisms of calcification. *Proc. Soc. Exper. Biol. & Med.*, 97: 688, 1958.
 345. CHEVILLARD, L. Sur les phosphomonoestérases de la moelle du fémur, des ganglions lymphatiques, des leucocytes d'exsudats chez le cobaye, et des plaquettes sanguines chez le lapin. *Compt. rend. Soc. de Biol.*, 139: 249, 1945.
 346. ZUCKER, M. B. and BORRELLI, J. A survey of some platelet enzymes and functions: the platelets as the source of normal serum acid glycerophosphatase. *Ann. New York Acad. Sc.*, 75: 203, 1958.
 347. ZUCKER, M. B. and BORRELLI, J. Platelets as a source of serum acid nitrophenylphosphatase. *J. Clin. Invest.*, 38: 148, 1959.
 348. KAY, H. D. Plasma phosphatase. I. Method of determination. Some properties of the enzyme. *J. Biol. Chem.*, 89: 235, 1930.
 349. ROCHE, J. Importance du substrat pour le pH optimum d'action des phosphatases sanguines (globules blanc et rouges, sérum). *Compt. rend. Soc. de Biol.*, 107: 640, 1931.
 350. ROCHE, J. Blood-phosphatases. *Biochem. J.*, 25: 1724, 1931.
 351. WACHSTEIN, M. Alkaline phosphatase activity in normal and abnormal human blood and bone marrow cells. *J. Lab. & Clin. Med.*, 31: 1, 1946.
 352. VALENTINE, W. N. The biochemistry and enzymatic activities of leukocytes in health and disease. *Progr. Haematol.*, 1: 293, 1956.
 353. FIESSINGER, N. and BOYER, F. La phosphatase plasmatique en pathologie hépatique. *Rev. méd. chir. mal du foie*, 10: 137, 1935.
 354. HAMPTON, J. C. An electron microscope study of the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the bile duct. *Acta Anat.*, 32: 262, 1958.
 355. BENNETT, H. S. The concepts of membrane flow and membrane vesiculations as mechanisms for active transport and ion pumping. *J. Biophys. & Biochem. Cytol.* 2 (suppl.): 99, 1956.

The Clinical Significance of Serum Acid Phosphatase*

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THE high phosphatase activity in acid solutions of human semen and of extracts of human prostatic tissue was first reported by Kutscher and Wolbergs [1] and Kutscher and Wörner [2]. These authors showed that the enzyme will hydrolyze a number of phosphate esters including phenyl phosphate and β -glycerophosphate, that it has a broad range of maximum activity between pH 4 and pH 6, that it is not activated by magnesium and is readily inhibited by fluoride.

During the next six years the clinical significance of prostatic acid phosphatase was studied extensively by the Gutmans and their co-workers [3-10], with results which were confirmed by others including myself [11-15]. While there was a good deal of variation in detail, the general picture which emerged from the results of these early studies was quite consistent and no serious discrepancies have been found in later work.

In summary, the results were as follows: Extracts of numerous tissues such as bone, liver and kidney were found to have slight acid phosphatase activity. The adult human prostate had up to one thousand times the activity of that of the other tissues examined, but this high activity did not appear until puberty. The prostate of the dog had moderate acid phosphatase activity, but the activity of the prostates of several other species was very small, and the enzyme thus did not appear to be of universal importance in the metabolism of the organ.

Because of its different pH of maximum activity and its different behavior toward fluoride and magnesium, prostatic acid phosphatase was shown to be distinct from alkaline phosphatases of various origins. It was also found to differ in many respects from the acid phosphatase of erythrocytes, but this difference was, and has remained, less clear-cut than that between acid and alkaline phosphatases. Since the enzyme was capable of hydrolyzing a wide spectrum of phos-

phate esters it was also evident that it was distinct from enzymes such as the hexose phosphatases which are highly specific for a single substrate. The King-Armstrong method for phenyl phosphatase and the Bodansky method for glycerophosphatase were modified by the Gutmans and by Woodard, respectively, for the determination of acid phosphatase in serum. These methods do not distinguish between acid phosphatase originating in the prostate and in other organs.

The clinical importance of prostatic acid phosphatase was first evident when it was discovered [3] that bones which were the site of metastases from prostatic carcinoma had an acid phosphatase activity up to one hundred times that of the corresponding normal bones. Very soon it was reported [4,5,11] that the serum of normal human subjects contained an acid phosphatase of unknown, non-prostatic origin, but that there was a greatly increased acid phosphatase activity in the serum of the majority of men with metastases in bone from prostatic carcinoma.

Determinations of serum acid phosphatase were adopted promptly as an aid in diagnosing prostatic carcinoma and in following the progress of the patients during the course of the various types of endocrine therapy which began to be employed extensively at about that time. It was soon found that, in patients whose serum acid phosphatase was elevated before treatment, there was usually a marked fall within three or four days after surgical castration, or within two weeks after the institution of estrogen therapy. In some patients the serum acid phosphatase reached normal limits; in others it remained slightly elevated, but much lower than the pre-treatment levels. Patients whose acid phosphatase did not decrease significantly during endocrine therapy failed to show satisfactory remissions clinically. After the results of longer periods of observation became available it was

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clear [16] that, when there had been a satisfactory fall in serum acid phosphatase during clinical remission, the readings remained low as long as the remission continued. If relapse occurred the acid phosphatase sometimes, but not always, rose again, but frequently failed to reach the pretreatment level even in the presence of widespread, active disease.

A brief digression to consider changes in serum alkaline phosphatase seems appropriate here. The serum alkaline phosphatase of men with metastases in bone from prostatic carcinoma is elevated more consistently than in patients with metastases in bone from any other primary, about 90 per cent of all untreated patients showing significant elevations at the first determination [13,16,17]. This correlates well with the brisk reparative process which takes place in the bone adjacent to the metastatic tumor and is shown radiographically by a marked osteoplasia. When such patients are given endocrine therapy the serum alkaline phosphatase commonly rises still higher, reaching a peak in about three months and then decreasing. In patients who do well the fall continues until near normal levels are reached; if relapse occurs the alkaline phosphatase tends to rise again. I have termed this phenomenon a "flare," and have discussed it extensively elsewhere [16,18]. It was described as early as 1941 [13], but has recently been reported [19] as a new finding peculiar to patients with prostatic carcinoma. It is not, however, confined to this disease, but is also seen frequently in women receiving endocrine therapy for mammary carcinoma metastatic to bone. Its exact cause is not known, but it is evident that it occurs at a time when a reduction in the rate of growth of the metastatic tumor permits more effective bone regeneration than was possible prior to treatment.

The relation between serum acid phosphatase and prostatic carcinoma, as shown by the early work previously mentioned, has in general been substantiated by later studies. Certain of these reports have, however, been misinterpreted, and the resulting misapprehensions have been perpetuated in the literature, and require discussion. A belief has grown up that the production of acid phosphatase by prostatic carcinoma is greater than that by the normal prostate, and that the enzyme appears in the blood stream only when metastasis has taken place to bone, but not when the deposits are confined to

the soft parts. In 1952 I showed [20] that the acid glycerophosphatase activity of extracts of prostatic carcinoma tissue from untreated patients was, on the average, considerably less than that from normal or hypertrophied prostatic tissue, although there was some overlapping. Prostatic carcinoma tissue from patients who had received prolonged endocrine therapy averaged only about one tenth of the activity of that from untreated patients. Additional material which I have obtained since this paper was published has confirmed this, as have scattered reports from other authors. At the same time I showed that the serum acid phosphatase of men with prostatic carcinoma correlated much better with the extent of the metastases than with their site. Elevations of serum acid phosphatase were very uncommon when the disease was confined to the gland, and occurred about as often in patients with metastases only in the soft parts as in those with metastases in bone. About one fourth of untreated patients with disseminated disease had no clear-cut elevation in serum acid phosphatase. The most probable explanation of these findings is that the intact prostate gland contains barriers which ordinarily prevent escape of acid phosphatase into the circulation. When metastasis takes place from prostatic carcinoma these barriers are no longer present, and prostatic secretion enters the circulation. Although most prostatic cancers elaborate less acid phosphatase than does the normal gland, they usually do produce enough so that the enzyme can be detected in the blood stream unless the normal barriers are still intact. Some untreated prostatic cancers produce too little acid phosphatase to be demonstrated in the serum, and, after prolonged endocrine therapy, the ability of the tumor to produce the enzyme is depressed so far that little is found in the serum even in the presence of extensive active disease. These relationships were suspected quite early [10,27], but were not established firmly until larger series of patients became available.

Serum Acid Phosphatase of Non-prostatic Origin.

It was observed early that the serum of women had a small but significant acid phosphatase activity, and that there was little difference between the activities of the serums of normal men and normal women. Likely sources of the enzyme in female serum were the liver and the erythrocytes, both of which, especially the latter, were known to have substantial acid phosphatase activities. Recently it has been shown [21,22]

that the blood platelets are the source of an acid phosphatase which is liberated during clotting. In addition to the acid phosphatase which is probably contributed from these sources to the serum of normal persons, it seems likely that acid phosphatase from some diseased tissues unrelated to the prostate may enter the circulation in measurable amounts. Occasional elevations in serum acid phosphatase have been reported [7,8,10,23] in women with Paget's disease of bone, metastases in bone, and hyperparathyroidism. It also appears [24,25] that such elevations occur frequently in Gaucher's disease. A few apparent elevations in serum acid phosphatase may have been due to lack of rigid control of the pH of reaction mixtures containing serums with very high alkaline phosphatase activities, since alkaline phosphatase may show some residual activity at a pH as low as 5.5, but, in many cases, this source of error was excluded. It is noteworthy that, in all of these clinical studies, phenyl phosphate was used as substrate. To date I have seen only a single elevation in serum acid phosphatase in a woman when glycerophosphate was used as substrate. This was in a patient with plasma cell myeloma who will be discussed later.

Properties of Acid Phosphatase. The discovery that elevations of serum acid phosphatase occurred occasionally in women and in men without prostatic disease led to attempts to develop a test which would be specific for prostatic phosphatase in serum. To this end numerous studies have been made of the properties of acid phosphatase of various origins. The most comprehensive study of this type is that of Abul-Fadl and King [26]. In this work both phenyl phosphate and glycerophosphate were used as substrates, and, in some cases, both the phenol and the phosphate ion resulting from the hydrolysis of phenyl phosphate were determined. The literature in this field is now too extensive to be reviewed in detail here, but references to some of the more important findings are given in Table 1. The reader is referred to the original articles for details of methods used, magnitude and reversibility of changes seen, etc. *

Prostatic acid phosphatase loses its activity very rapidly when heated to 37°C. in slightly alkaline solution. The acid phosphatases from

several other sources are similarly, although not quite so rapidly, inactivated; the acid phosphatase of blood platelets appears to be somewhat more stable in this respect. The instability of prostatic acid phosphatase at physiological temperature and pH is great enough to account for the known rapid disappearance of the enzyme from the circulation. Ethyl alcohol and L-tartrate appear to have a rather highly selective inhibitory effect on prostatic acid phosphatase, and little effect on the acid phosphatase of erythrocytes. The reverse is true of cupric ions and formaldehyde which inhibit erythrocyte phosphatase very markedly and prostatic phosphatase very little. The references cited in the table, as well as the report of Abul-Fadl and King, show, however, that the effects of these agents are not entirely specific, most of them having some effect on the activities of the acid phosphatase of the serum of women and of extracts of liver, kidney and spleen.

The differences found in the way in which acid phosphatase preparations of different origins are affected by various added substances, as well as in the shape of their pH-activity curves and their relative activities toward different substrates, have led some authors to conclude that the prosthetic groups are different. This may be so, and, in particular, the acid phosphatase of erythrocytes differs rather conspicuously from that of several other organs. It must be remembered, however, that, to date, no phosphatase has been crystallized, although some workers have obtained highly purified preparations [34b]. The elegant work of Schlamowitz [35,36] has shown that alkaline phosphatase, even when highly purified, retains the immunological reactions of the species and organ of origin. It seems likely, although not yet definitely proved, that acid phosphatase is similarly closely bound to proteins characteristic of the tissue of origin. In crude preparations, or in the blood serum, the enzyme is likely to be associated with still larger protein complexes capable of reacting with enzyme, substrate and inhibitor in ways which would cause substantial modifications of activity. In the absence of specific proof that this is not so the hypothesis that different tissues elaborate distinct acid phosphatase prosthetic groups remains unproved.

Tests for Prostatic Acid Phosphatase in Serum. While no completely specific differences between the acid phosphatases of the prostate and of other organs were demonstrated in the work

* In the table and elsewhere in this paper the word "inhibition" is used loosely to indicate any conditions in which enzyme activity is decreased. In much of the work cited it was not known or not stated whether or not the process was reversible.

TABLE I
INHIBITION OF ACID PHOSPHATASES

| Inhibitor | Substrate | Inhibition of Prostatic Acid Phosphatase | Inhibition of Non-prostatic Acid Phosphatase | References |
|---|--------------------------------|--|--|------------|
| 37°C. temperature and alkaline pH <i>in vitro</i> | β -glycerophosphate | | Slight inhibition of platelet acid phosphatase | [27] |
| | <i>p</i> -nitrophenylphosphate | | Slight inhibition of platelet acid phosphatase | [22] |
| | Phenyl phosphate | Marked | Minimal effect on non-prostatic acid phosphatase of serum | [27] |
| | β -glycerophosphate | Marked | Moderate to marked inhibition of acid phosphatase of rat liver or female serum | [28] |
| <i>In vivo</i> disappearance probably due to temperature and pH effects | Phenyl phosphate | Fall in serum acid phosphatase during fever | | [14] |
| | β -glycerophosphate | Dependence of serum acid phosphatase on body temperature | | [29] |
| | β -glycerophosphate | Disappearance from serum of acid phosphatase expressed from prostate | | [30] |
| Ethyl alcohol 40% | Phenyl phosphate | Marked | Minimal effect on non-prostatic acid phosphatase of serum | [27] |
| Formaldehyde 2% | Phenyl phosphate | Minimal | Marked inhibition of erythrocyte acid phosphatase | [31] |
| | Phenyl phosphate | Minimal | Moderate inhibition of acid phosphatase of spleen, liver and kidney | [32] |
| L(+) Tartaric acid, 0.02 M | Phenyl phosphate | Almost complete | Minimal inhibition of erythrocyte acid phosphatase | [32] |
| | Phenyl phosphate | Almost complete | Some effect on acid phosphatase of female serum | [33] |
| Cupric sulfate, 0.001 M | Phenyl phosphate | Minimal | Marked inhibition of erythrocyte acid phosphatase. Some inhibition of acid phosphatase of spleen, liver and kidney | [34] |

summarized in Table I, some of the differences found were sufficiently marked to warrant making them the basis of tests which gave promise of clinical usefulness. The first such attempt was made by Herbert [27]. By measurement of the fraction of the serum total acid phosphatase which was inactivated by treatment with ethyl alcohol for a half hour at room temperature she was able to demonstrate abnormal readings in the serum of a somewhat larger proportion of

patients with known prostatic carcinoma than were found in similar serums without alcohol treatment. Delory et al. [37] and Kintner [37] made parallel determinations of the effects of ethyl alcohol and of formaldehyde in the serums of a large series of normal men and of patients with a variety of diseases including treated and untreated prostatic carcinoma. In Delory's series, of 209 patients with diseases other than prostatic carcinoma thirty-seven had total acid

phenyl phosphatase readings above the range found in normal men; the formaldehyde-resistant enzyme was increased in only sixteen of these. They conclude that the formaldehyde method may be useful in clarifying the diagnosis in some doubtful cases, and has the advantage of simplicity, but is not entirely specific. They did not find the alcohol method useful.

Lemon and his co-workers [34a,38] have studied the copper-resistant serum acid phenyl phosphatase in a large series of normal subjects and patients with various diseases. In addition to the expected elevations which were found in men with prostatic carcinoma, they also found elevations in both men and women with other types of cancer, benign tumors, and non-neoplastic diseases. They believe that determinations of copper-resistant serum acid phosphatase are as useful in following the course of mammary cancer as that of prostatic cancer. Many of the differences which they found between the groups of subjects are significant statistically, but there is sufficient overlapping so that the method does not seem to be specific enough to be reliable in diagnostic work. The findings are quite important theoretically, as they indicate that, in a wide variety of diseased states, the serum has an increased capacity to split phenyl phosphatase under conditions which largely prevent the activity of the phosphatase of erythrocytes. Unfortunately the clinical data given are insufficient to permit a correlation between serum enzyme activity and the organs, such as marrow, bone, liver, etc., which were involved secondarily in the diseases concerned.

The inhibition caused by L-(+)-tartrate is highly, although not completely, specific for acid phenyl phosphatase of prostatic origin, and has been used extensively in clinical procedures designed to demonstrate the presence of small amounts of prostatic phosphatase in the blood serum. Fishman and his associates [39-42] have reported considerable success in demonstrating increases in prostatic acid phosphatase in the serum after massage of the prostate, and in men with prostatic carcinoma whose serum total acid phosphatase was within normal limits. They consider that elevations in the tartrate-inhibited fraction of the enzyme in the serum indicate the presence of occult prostatic cancer even when this is not detectable clinically. Some doubt is thrown on this hypothesis, however, by the fact that tartrate-inhibited, or so-called "prostatic," acid phosphatase occurs in the serum of women,

and is increased in some diseases. Other investigators [43-45] report that they have found the method to be useful occasionally in demonstrating true prostatic enzyme in the circulation, but do not feel that its superiority to the usual determination of serum total acid phosphatase has been demonstrated clearly.

Sources of Serum Acid Phosphatase. The work thus far reviewed shows that it is possible to distinguish fairly clearly between the acid phosphatase of the erythrocytes and that of the prostate. The serum of both normal and abnormal men and women contains measurable acid phosphatase activity which does not have the characteristics of erythrocyte phosphatase, which cannot be distinguished clearly from prostatic phosphatase, and whose origin is, at present, unknown. For both theoretical and practical reasons it is desirable to know how this enzyme varies in health and disease. It is also important to know how often prostatic acid phosphatase enters the circulation of men without prostatic disease.

In the numerous reports which have appeared on the results of determinations of the total or fractional acid phenyl phosphatase of the serum of women or of men with normal prostates, from 2 per cent to 10 per cent of the readings have been above the limits accepted as normal in the particular study concerned. Because of differences in experimental method, clinical material, and definition of normal range, it is difficult to compare the results of the different studies in detail. It is clear, however, that the serum of significant numbers of patients with disease of bone, liver, and some other organs, contains more of an enzyme which will split phenyl phosphate in acid solution than does the serum of healthy persons.

In a previous paper [20], using beta glycerophosphate as substrate, I reported serum acid phosphatase readings in a series of 492 subjects, including normal men and women and patients with various diseases unrelated to the prostate. This series has now been increased to include 878 persons, of whom thirty-five women and sixty-five men were normal. Of the abnormal cases, about 10 per cent had osteogenic sarcoma, and 15 per cent had Paget's disease of bone. The remainder were divided nearly equally between patients with metastatic disease in bone, various types of benign or malignant primary bone tumors other than osteogenic sarcoma, and various diseases unrelated to bone but often

involving the liver. A detailed analysis of the findings in the different groups seems unnecessary, since, while some differences were found in the means of the acid phosphatase readings, these differences were all less than the corresponding standard deviations. The results for all the males and for all the females are presented graphically in Figure 1.

For the females the mean serum acid phosphatase was 0.39 units/100 cc., standard deviation 0.19; for the males the mean was 0.40, standard deviation 0.21. In this discussion readings above 0.90 units/100 cc. will be considered abnormal. The figure of 0.90 is slightly more than two standard deviations above the mean of 0.42 found for normal men, and slightly above the highest reading found in normal women. In the entire series readings higher than this were found in one woman and six men. This incidence of 0.8 per cent is much lower than the incidence of elevated acid phosphatase readings in the serum of patients without prostatic disease which has been reported by many of the investigators who used phenyl phosphate as substrate. According to statistical theory, 2.3 per cent of a series of determinations may be expected to exceed the mean by more than two standard deviations, and the 0.8 per cent found here can thus be considered to be due solely to random variation. In all biological work, however, it is desirable to inquire whether such variation can reasonably be attributed solely to laboratory error, or whether it is due to non-random, but unrecognized, variations in the material studied.

Five of the men in the series had acid phosphatase readings between 0.94 and 1.3 units/100 cc. Three of these, all suffering from osteogenic sarcoma, had one or more normal readings during the few weeks subsequent to the initial elevated one. One, a youth of twenty years, was found to have had a rectal examination immediately before the first blood sample was drawn, and the possibility of prior rectal examination could not be excluded in the other two cases. In one man with Paget's disease of bone and one with plasma cell myeloma it was not possible to obtain blood for additional determinations. While it is not possible to exclude experimental error as a cause of the minor elevations in serum acid phosphatase in these five men, it seems almost certain in one case, and likely in two others, that the elevations were due to transitory conditions which permitted escape of prostatic phosphatase into the circulation.

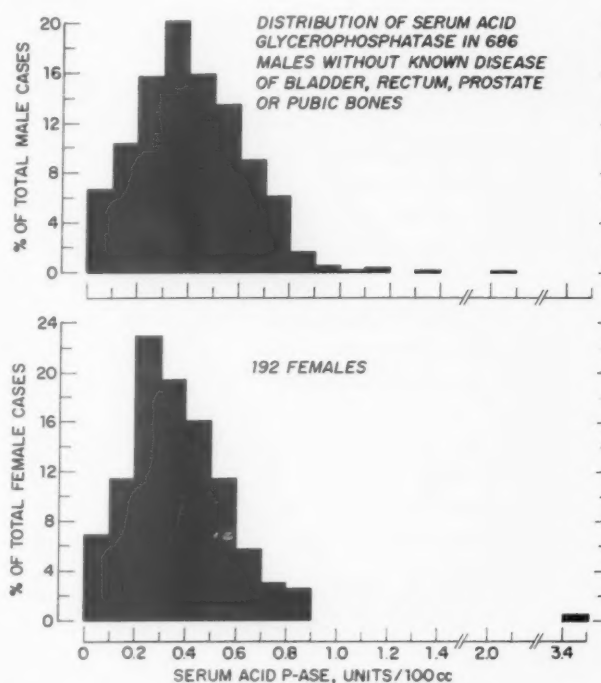


FIG. 1. Distribution of serum acid glycerophosphatase readings in normal men and women and in patients with diseases unrelated to the prostate gland.

This is not true of two patients in whom definite and persistent elevations in serum acid phosphatase were found. One of the patients was a man, aged forty-four, with a small, non-specific inflammatory lesion of the lower right tibia. This was curetted in October 1956 and failed to heal either per primum or after four attempts at skin grafting. On nine occasions between October 1956 and March 1959 his serum acid phosphatase was found to range between 1.8 and 3.6 units/100 cc. He underwent several thorough urological examinations, and no evidence of prostatic disease was found. Other systems also appeared normal, and his general health was good. He is being watched closely for the development of metabolic disturbances such as Gaucher's disease.

The other patient with a persistent elevation in serum acid phosphatase was a woman, aged fifty-one, with advanced plasma cell myeloma, confirmed by biopsy. She had received extensive x-ray therapy, including whole-body radiation. Her serum acid phosphatase ranged from 1.8 to 12.8 units/100 cc. in eight determinations made over a period of three months.

The source of acid phosphatase in the serum of these patients and of the numerous patients without prostatic disease who have been reported to have elevations in serum acid phenyl phos-

phatase remains unknown. It seems reasonable to suppose that the enzyme is elaborated by various types of abnormal tissue, or that during periods of stress it is produced in increased amounts, or enters the circulation more readily, from various normal tissues which are known to contain it. It is to be hoped that studies will be made of the acid phosphatase activities toward several substrates of plasma cell myeloma tissue and the tissue of Gaucher's disease. I have not seen published reports of such studies. In a single unpublished determination I found 3.3 units/gm. of acid glycerophosphatase in a specimen of plasma cell myeloma tissue removed by biopsy. An activity as high as this might account for elevated readings in the serum of patients with extensive disease such as the cases reported here. That this situation is not common is suggested by the fact that I found normal acid phosphatase activities in the serum of fifty-five other patients with plasma cell myeloma, some of whom had repeated determinations made for several years. In extracts of various types of normal and abnormal bone I have noted [46] some acid glycerophosphatase activity, but have not found elevations in the serum of patients with primary bone disease, with the doubtful exceptions discussed above. Elevations in acid phenyl phosphatase have been found frequently in the serum of such patients, and this has led to the suggestion that the acid phosphatase elaborated by bone is relatively inactive toward β -glycerophosphate. It would be highly desirable to test this hypothesis by making parallel determinations of the activities toward both substrates of the serums of a large series of patients, preferably women, with various types of disease of bone.

In addition to identifying non-prostatic sources of acid phosphatase in the serum, it is important to know how often prostatic phosphatase enters the circulation of men without prostatic disease. It is well established that mild trauma to the prostate, such as that resulting from massage or catheterization, will cause transitory elevations in serum acid phosphatase. In one fairly extensive study [47] such elevations were not found, but heparinized blood was used in the work, and this may have influenced the results, as heparin inhibits prostatic acid phosphatase to some extent. Except in this one study most workers have reported at least sporadic abnormal readings in the serum after mild prostatic trauma, and it is desirable to know

whether similar elevations occur in the serum of normal men as a result of everyday activities.

In the data which are summarized in Figure 1 no elevations were found in the acid phosphatase of the serum of the sixty-five men who were normal, but the number of subjects in this group is too small for evaluation of the significance of negative findings. Day et al. [48] have reported the results of determinations of total and tartrate-inhibited acid phosphatase in the serum of 315 men aged twenty to seventy-nine years with clinically normal prostates. Of these 315 men, eighteen, or 5.7 per cent, had either total or tartrate-inhibited acid phosphatase readings higher than two standard deviations above the mean for the group. This incidence is higher than that to be expected by chance. In most of these subjects it was possible to make subsequent determinations, the results of which were all within the normal range. The number of subjects in this series is large, and patients with clinical evidence of prostatic disease were excluded with unusual care. Hence the conclusion seems justified that small and transitory elevations in total or fractional serum acid phenyl phosphatase occur in significant numbers of normal men. This finding tends to diminish the reliability for the diagnosis of prostatic carcinoma of tests designed to detect small amounts of prostatic phosphatase in the serum, at least as far as single determinations are concerned. Persistent elevations, even when of small magnitude, do, however, seem [45] to be a fairly reliable indication of prostatic carcinoma. It is, of course, generally accepted that grossly abnormal readings are highly significant in diagnosis.

SUMMARY

The history of the development of tests for serum acid phosphatase and of the use of such tests in the diagnosis and treatment of patients with prostatic carcinoma is reviewed. Most of the early work is found to be reliable when evaluated in the light of subsequent experience.

The properties of the acid phosphatases originating in various organs and the methods of distinguishing between them are discussed. The evidence cited indicates that it is possible to distinguish fairly clearly between the acid phosphatase of the prostate and that of the erythrocytes, but differences between prostatic phosphatase and the acid phosphatase from some other sources appear to be less clear-cut.

The theoretical basis of several methods for

the determination of serum acid phosphatase is outlined.

Evidence is cited which indicates that men with normal prostates and also women may show elevations in serum acid phosphatase if they suffer from disease of any one of several organs. The incidence of such elevations varies with the experimental method, and is less frequent when β -glycerophosphate is used as substrate than when some other substrates are employed. Suggestions are made as to methods by which the origin of this enzyme activity might be clarified.

Evidence is also cited which indicates that the possibility of the spontaneous transitory appearance of prostatic acid phosphatase in the serum of normal men should be considered in evaluating the significance of serum acid phosphatase readings.

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REFERENCES

1. KUTSCHER, W. and WOLBERGS, H. Prostatic phosphatase. *Ztschr. f. physiol. Chem.*, 236: 237, 1935.
2. KUTSCHER, W. and WÖRNER, A. Prostatic phosphatase. II. *Ztschr. f. physiol. Chem.*, 239: 109, 1936.
3. GUTMAN, E. B., SPROUL, E. E. and GUTMAN, A. B. The significance of increased phosphatase activity of bone at the site of osteoplastic metastases secondary to carcinoma of the prostate. *Am. J. Cancer*, 28: 485, 1936.
4. GUTMAN, A. B. and GUTMAN, E. B. "Acid" phosphatase activity of the serum of normal human subjects. *Proc. Soc. Exper. Biol. & Med.*, 38: 470, 1938.
5. GUTMAN, A. B. and GUTMAN, E. B. An "acid" phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostate gland. *J. Clin. Invest.*, 17: 473, 1938.
6. GUTMAN, A. B. and GUTMAN, E. B. "Acid" phosphatase and functional activity of the prostate (man) and preputial glands (rat). *Proc. Soc. Exper. Biol. & Med.*, 39: 529, 1938.
7. ROBINSON, J. N., GUTMAN, E. B. and GUTMAN, A. B. Clinical significance of increased serum "acid" phosphatase in patients with bone metastases secondary to prostatic carcinoma. *J. Urol.*, 42: 602, 1939.
8. GUTMAN, A. B., GUTMAN, E. B. and ROBINSON, J. N. Determination of serum "acid" phosphatase activity in differentiating skeletal metastases secondary to prostatic carcinoma from Paget's disease of bone. *Am. J. Cancer*, 38: 103, 1940.
9. GUTMAN, E. B. and GUTMAN, A. B. Estimation of "acid" phosphatase activity of blood serum. *J. Biol. Chem.*, 136: 201, 1940.
10. (a) SULLIVAN, T. J., GUTMAN, E. B. and GUTMAN, A. B. Theory and application of the serum "acid" phosphatase determination in metastasizing prostatic carcinoma: early effects of castration. *J. Urol.*, 48: 426, 1942.
(b) GUTMAN, A. B. Serum "acid" phosphatase in patients with carcinoma of the prostate gland, present status. *J. A. M. A.*, 120: 1112, 1942.
11. BARRINGER, B. S. and WOODARD, H. Q. Prostatic carcinoma with extensive intraprostatic calcification. *Tr. Am. A. Genito-urin. Surgeons*, 31: 363, 1939.
12. DEAN, A. L., WOODARD, H. Q. and TWOMBLY, G. H. The endocrine treatment of cancers of the prostate. *J. Urol.*, 49: 108, 1943.
13. HUGGINS, C. and HODGES, C. V. Studies on prostatic cancer. I. The effect of castration, of estrogen and of androgen injections on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res.*, 1: 293, 1941.
14. HUGGINS, C., SCOTT, W. W. and HODGES, C. V. Studies on prostatic cancer. III. The effects of fever, of desoxycorticosterone and of estrogen on clinical patients with carcinoma of the prostate. *J. Urol.*, 46: 997, 1941.
15. WOODARD, H. Q. Acid and alkaline glycerophosphatase in tissue and serum. *Cancer Res.*, 2: 497, 1942.
16. WOODARD, H. Q. and DEAN, A. L. The significance of phosphatase findings in carcinoma of the prostate. *J. Urol.*, 57: 158, 1947.
17. WOODARD, H. Q. Changes in blood chemistry associated with carcinoma metastatic to bone. *Cancer*, 6: 1219, 1953.
18. WOODARD, H. Q., ESCHER, G. C. and FARROW, J. H. Changes in the blood chemistry of patients with disseminated carcinoma of the breast during endocrine therapy. *Cancer*, 7: 744, 1954.
19. WRAY, S. The significance of the blood acid and alkaline phosphatase values in cancer of the prostate. *J. Clin. Path.*, 9: 341, 1956.
20. WOODARD, H. Q. Factors leading to elevations in serum acid glycerophosphatase. *Cancer*, 5: 236, 1952.
21. ZUCKER, M. B. and BORRELLI, J. A survey of some platelet enzymes and functions: the platelets as the source of normal serum acid glycerophosphatase. *Ann. New York Acad. Sc.*, 75: 203, 1958.
22. ZUCKER, M. B. and BORRELLI, J. Platelets as a source of serum acid nitrophenyl-phosphatase. *J. Clin. Invest.*, 38: 148, 1959.
23. KLENDSHOJ, N. C. and KOEFF, G. F. Serum phosphatase activity in hyperparathyroidism. *J. Clin. Endocrinol.*, 3: 351, 1943.
24. TUCHMAN, L. R., SUNA, H. and CARR, J. J. Elevation of serum acid phosphatase in Gaucher's disease. *J. Mount Sinai Hosp.*, 23: 227, 1956.
25. TUCHMAN, L. R. and SWICK, M. High acid phosphatase level indicating Gaucher's disease in patient with prostatism. *J. A. M. A.*, 164: 2034, 1957.
26. ABUL-FADL, M. A. M. and KING, E. J. Properties of the acid phosphatases of erythrocytes and of the human prostate gland. *Biochem. J.*, 45: 51, 1949.
27. HERBERT, F. K. The estimation of prostatic phosphatase.

- tase in serum and its use in the diagnosis of prostatic carcinoma. *Quart. J. Med.*, 39: 221, 1946.
28. WOODARD, H. Q. A note on the inactivation by heat of acid glycerophosphatase in alkaline solution. *J. Urol.*, 65: 688, 1951.
 29. LONDON, M., MCHUGH, R. and HUDSON, P. B. Low acid phosphatase values of patients with known metastatic cancer of the prostate. *Cancer Res.*, 14: 718, 1954.
 30. WHITMORE, W. F. and WOODARD, H. Q. The effects of prostatic surgery on the level of serum acid glycerophosphatase. *J. Urol.*, 74: 809, 1955.
 31. DELORY, G. E., SWEETSER, T. H. and WHITE, T. A. The use of formalin and alcohol in the estimation of prostatic phosphatase. *J. Urol.*, 66: 724, 1951.
 32. DELORY, G. E. and HETHERINGTON, M. The acid phosphatase of human urine. *Canad. J. M. Sc.*, 30: 4, 1952.
 33. FISHMAN, W. H. and LERNER, F. A method for estimating serum acid phosphatase of prostatic origin. *J. Biol. Chem.*, 200: 89, 1953.
 34. (a) REYNOLDS, M. D., LEMON, H. M. and BYRNES, W. W. Copper-resistant serum acid phosphatase. I. Method and values in health and disease. *Cancer Res.*, 16: 943, 1956.
(b) LONDON, M. and HUDSON, P. B. Studies on the purification of acid prostatic phosphatase. *Arch. Biochem.*, 46: 141-153, 1953.
 35. SCHLAMOWITZ, M. Production of antibodies against dog intestinal phosphatase. *J. Biol. Chem.*, 206: 361, 1954.
 36. SCHLAMOWITZ, M. and BODANSKY, O. Tissue sources of human serum alkaline phosphatase as determined by immunochemical procedures. *J. Biol. Chem.*, 234: 1433, 1959.
 37. KINTNER, E. P. The estimation of prostatic phosphatase using a modified King-Armstrong method by determining the formaldehyde stable and alcohol labile fraction of serum acid phosphatase. *J. Lab. & Clin. Med.*, 37: 637, 1951.
 38. LEMON, H. M. and REYNOLDS, M. D. Regression of mammary cancer metastases following decline of serum acid phosphatase induced by anti-estrogen therapy. *Proc. Am. A. Cancer Res.*, 2: 129, 1956.
 39. FISHMAN, W. H., DART, R. M., BONNER, C. D., LEADBETTER, W. F., LEARNER, F. and HOMBURGER, F. A new method for estimating serum acid phosphatase of prostatic origin applied to the clinical investigation of cancer of the prostate. *J. Clin. Invest.*, 32: 1034, 1953.
 40. FISHMAN, W. H., BONNER, C. D. and HOMBURGER, F. Some factors which influence the level of the serum "prostatic" acid phosphatase. *Proc. Am. A. Cancer Res.*, 1: 14, 1954.
 41. FISHMAN, W. H., BONNER, C. D. and HOMBURGER, F. Serum "prostatic" acid phosphatase and cancer of the prostate. *New England J. Med.*, 225: 925, 1956.
 42. FISHMAN, W. H., DAVIDSON, H. M., BONNER, C. D. and HOMBURGER, F. Studies on prostatic acid phosphatase and cancer of the prostate. *Proc. Am. A. Cancer Res.*, 2: 105, 1956.
 43. HILL, J. H. Prostatic serum acid phosphatase in patients with localized prostatic cancer. *Am. J. Clin. Path.*, 26: 120, 1956.
 44. NOBLES, E. R., KERR, W. S., DUTOIT, C. H. and ROURKE, G. M. Serum prostatic acid phosphatase levels in patients with carcinoma of the prostate. *J. A. M. A.*, 164: 2020, 1957.
 45. WHITMORE, W. F., BODANSKY, O., SCHWARTZ, M. K., YING, S. H. and DAY, E. Serum prostatic acid phosphatase levels in proved cases of carcinoma or benign hypertrophy of the prostate. *Cancer*, 9: 228, 1956.
 46. WOODARD, H. Q. Quantitative studies of beta glycerophosphatase in normal and neoplastic tissues. *Cancer*, 9: 352, 1956.
 47. DYBKAER, R. and JENSEN, G. Acid serum-phosphatase levels following massage of the prostate. *Scandinav. J. Clin. & Lab. Invest.*, 10: 349, 1958.
 48. DAY, E., YING, S. H., SCHWARTZ, M. K. and BODANSKY, O. Serum prostatic acid phosphatase levels in the male patients of a cancer-prevention clinic. *Cancer*, 9: 222, 1956.

The Clinical Significance of Transaminase Activities of Serum*

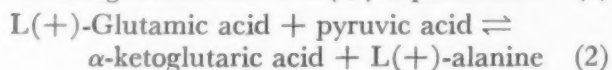
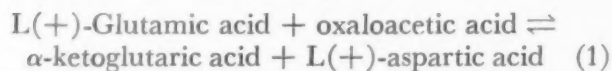
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FOLLOWING the demonstration of the normal range of activity of transaminase in human serum [75], a sizable experience has accrued attesting to the clinical implications of alterations in serum transaminase activity in diseases of the heart, liver, biliary tract and skeletal muscle [19,20,47,105,115,169,172]. It is the purpose of this review to point up the pertinent experimental studies which bear on the mechanisms of alterations in serum transaminase activity, and to review the observations made in the past several years which indicate the clinical significance of alterations in transaminase activity of the serum and cerebrospinal fluid.

Transamination. The intermolecular transfer of an amino group from an α -amino acid to an α -keto acid is an example of a transamination reaction. This may occur non-enzymatically, but a new concept in amino acid metabolism was introduced when enzymatic transamination was described, using pigeon breast muscle as a source of transaminase [16,17]. Enzymatic transamination, unlike non-catalyzed transamination, results in oxidative deamination without decarboxylation. It was first suggested that the tissue enzyme which catalyzed transamination reactions be called aminopherase [15], but later the name transaminase was suggested for the enzyme and this term is now generally employed.

With the utilization of tissue extracts, it was shown that enzymatic transamination is largely limited to two reactions [30,32]:



Indications of the existence of a coenzyme activator for the apotransaminase were sup-

ported by the evidence obtained from experimental studies of vitamin B₆ deficiency. Deficiency of vitamin B₆ was associated with lowered levels of transaminase activity, and the addition of pyridoxal phosphate to the tissue or cell preparations resulted in at least partial restoration of enzyme activity [3,7,99,151]. Recent studies have confirmed earlier work on the activation of transaminases by pyridoxal phosphate, and have shown as well that many of the newly discovered transaminases also require this coenzyme [110].

With the utilization of pyridoxal phosphate, it was demonstrated that muscle homogenates are capable of catalyzing the transfer of α -amino groups from twenty-five different amino acids and that this activity was present in pig heart, liver and kidney tissue homogenates [25], and in varying degrees of activity in eight organs of the rat [6,32].

The variety of amino and keto acids capable of participating in transamination makes it necessary to distinguish the different catalytic effects. Accordingly, it has become customary to distinguish the enzymes responsible for transamination reactions in one of several ways. The enzymes which catalyze reversible transamination reactions are designated by a term referring to both amino acids concerned, e.g., glutamate-aspartate transaminase. Another method of designating the enzymes is to name them by a hyphenated term describing the favored products of the equilibrium reaction, e.g., glutamic-oxaloacetic transaminase (GO-T) and glutamic-pyruvic transaminase (GP-T). The latter method of designation has been used in the clinical literature.

Although it now appears that at least several distinct transaminases exist in each of the tissues that have thus far been studied, final character-

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ization of the individual catalytic systems has yet to be achieved [110]. However, the chemical separation and purification of the GP-T and GO-T of heart muscle have been reported by several groups [23,24,62,71,98,131,150]. Both these enzymes have also been separated from each other electrophoretically, using as an enzyme source serum obtained from patients with hepatitis [171]. It would thus appear that at least GP-T and GO-T are separable, distinct, and individual catalytic systems.

Methods of Measuring Serum Transaminase Activity. Although a number of transaminase activities have been demonstrated in human serum [106], the two serum transaminases currently of clinical import are serum glutamic-oxaloacetic transaminase (SGO-T) and serum glutamic-pyruvic transaminase (SGP-T). Both serum enzymes have been measured chromatographically [75,76], spectrophotometrically [67,76,126,161,180], fluorometrically [95,96] and colorimetrically [22,164,176].

When aspartate or alanine is incubated with α -ketoglutarate and a source of enzyme, the rate of production of glutamate may be taken as a measure of transaminase activity. The amount of glutamate produced after a given incubation period under standardized conditions can be measured by quantitative paper chromatography [6,77]. Addition of a buffered solution of pyridoxal phosphate in a concentration of 10 μ g. per ml. was found to have no measurable effect on the transaminase activity of normal serum [76] but recent observations indicate that in some instances abnormally increased serum transaminase activity may be enhanced by the addition of pyridoxal phosphate [175]. In terms of glutamate formation, the SGO-T activity in normal man was found to vary from 0.41 to 1.36 μ M. glutamate formed per ml. serum per hour of incubation, with a mean activity of 0.622 ± 0.191 . The serum glutamic-pyruvic transaminase was found to vary between 0.21 and 1.01 per ml. serum per hour, with a mean value of 0.525 ± 0.146 .

A spectrophotometric method was devised in which the glutamic-oxaloacetic transamination reaction is coupled to the oxidation of reduced diphosphopyridine nucleotide (DPNH) by oxaloacetate in the presence of an excess of purified malic dehydrogenase. The oxidation of DPNH, and thereby the transamination reaction, is followed by measuring the decrease in light absorption at 340 $m\mu$., at which wavelength the

reduced pyridine nucleotides have an absorption peak. Serum glutamic-oxaloacetic transaminase activity is expressed as units per ml. serum per minute. One unit equals a decrease in optical density of 0.001 under standardized conditions [74]. At 23°C., the GO-T activity of serums of normal human adults was found to range between 9 and 40 units per ml. serum per minute, with a mean value of 20 ± 7 units.

The spectrophotometric measurement of SGP-T is accomplished by utilizing a technic analogous to that described for SGO-T [180]. The transamination reaction is coupled to the reduction of pyruvate to lactate by DPNH in the presence of an added excess of purified lactic dehydrogenase. Serum activity is expressed in units per ml. per minute. One unit equals a decrease in optical density of 0.001 under standardized conditions. Serum glutamic-pyruvic transaminase, measured at 23°C. in serums of normal human subjects, was found to have a mean activity of 16 ± 9 units per ml. per minute.

Several spectrophotometric technics and modifications for the measurement of SGO-T and SGP-T have been described [13,67,68,73,126,139,159,167]. The spectrophotometric technic lends itself to micromethodology [69,95,96,152,175].

A fluorometric method for measuring SGO-T and SGP-T has been described [95,96]. The coupled reactions used in the spectrophotometric technics are employed. However, instead of measuring the decrease in light absorption at wave length 340 $m\mu$. as DPNH is oxidized, the fluorescence of the condensation product of DPN with methyl-ethyl ketone is determined. The enzyme activity is expressed as the number of micromoles of oxaloacetate or pyruvate produced per hour per ml. of serum. Serum glutamic-oxaloacetic transaminase, measured at 37°C. in serums of normal human subjects, was found to have a mean activity of 1 ± 0.05 units per hour per ml. and SGP-T to have a mean activity of 0.8 ± 0.5 units.

The colorimetric method estimates serum transaminase activity by measuring the amount of pyruvate formed under standard conditions [22,44,50,65,115,136,176]. A technic used for the assay of GO-T in animal tissues has been modified and adapted to the measurement of SGO-T and SGP-T [162]. Measurement of the SGO-T activity involves the conversion of the formed oxaloacetate to pyruvate. The intensity of the color developed, using dinitrophenylhydrazine,

is proportional to the amount of pyruvate, and the quantity of pyruvate reflects the degree of SGO-T activity. Essentially the same principles are involved in the estimation of SGP-T, except that pyruvate is formed directly by transamination. One colorimetric unit of SGO-T or of SGP-T is defined as the activity of 1.0 ml. of serum that results in the formation of chromogenic material equivalent to 1 μ g. of pyruvate under standardized conditions. Spectrophotometrically, the measurement of serum transaminase activity depends on the rate of the transamination reaction rather than on the amount of a product formed during the enzymatic reaction. Actually, the two methods measure different aspects of transamination and are therefore not entirely comparable. One unit of transaminase activity measured colorimetrically is approximately equivalent to 1 unit estimated spectrophotometrically in the normal range; in the abnormal range of serum transaminase activity, 1 colorimetric unit approximates 2 spectrophotometric units. The mean SGO-T activity of serums of normal persons determined by the colorimetric technics is 16 ± 8.0 units, with a normal range of 4 to 40 units, while that of the SGP-T is 22 ± 12 units, with a normal range of 1 to 45 units. The principal advantage of the colorimetric method for serum transaminase assay is that it does not require ultraviolet spectrophotometry.

The stability of these serum enzymes is such as to facilitate their clinical usefulness. Storing at room temperature for twenty-four hours or at 4°C. for five days does not significantly alter serum transaminase activity. Serum glutamic-oxaloacetic transaminase and SGP-T determinations can be made without regard to the fasting state [28,76,167]. The mechanism for excretion and/or degradation of serum transaminase is unknown. It is pertinent, however, that oliguria and/or azotemia are not necessarily associated with elevated serum transaminase activity [48,92,179].

Tissue Distribution of Transaminase Activity. The transaminase activities of different tissues vary, with distinct species differences [15, 31,35,55,85,91,110,119,127,140]. In all instances the activity of GO-T in any one tissue is greater than that of GP-T. In the case of GO-T, the greatest activity is observed in extracts of skeletal, diaphragm and heart muscle, and of liver tissue. The relative distribution of transaminase in tissue homogenates of the normal

adult has been reported [180]. The impressive transaminase activity of tissue homogenates, as contrasted with the relatively minute activity of serum, suggested that tissue injury might be associated with increments in serum transaminase activity.

ALTERATIONS OF SERUM TRANSAMINASE IN CARDIAC DISEASE

It was observed that during the first several days following transmural myocardial infarction in man, SGO-T is increased above the normal range [76], an observation subsequently confirmed and extended [10,21,27,28,45,58,60,66, 77,78,80,81,89,90,92,93,100,113,147,160,170].

Various technics have been used in the study of experimentally produced myocardial infarction [57,127,133,141,142,144]. Coronary occlusion was produced by coronary artery ligation in the closed-chest dog, and it was found that alterations in SGO-T correlated with electrocardiographic and other observations [127]. The rise of the enzyme in the serum, as well as the duration of the rise, roughly is proportional to the extent of infarction of the heart muscle. These observations were strikingly similar to those previously reported following myocardial infarction in man. The sensitivity of SGO-T as a reflection of cardiac muscle necrosis is demonstrated by the fact that infarcts less than 1 gm. in size resulted in significant although short-lived elevations of SGO-T. The fact that the activity of GO-T in infarcted muscle is appreciably less than that in the adjacent normal muscle of the same heart, and the observation that the GO-T activity in infarcted muscle diminishes proportionately with the age of the infarct, suggest that part of the mechanism of elevation of the SGO-T is release of intracellular enzyme into the blood stream following death of the cell or loss of integrity of the cellular membrane [112,127,142].

Myocardial ischemia of varying duration has been produced in dogs by means of temporary occlusion of coronary arteries, and the degree of ischemia correlated with electrocardiographic changes, SGO-T and SGP-T alterations, and heart tissue GO-T and GP-T activities [128,143, 175]. With a few exceptions, myocardial ischemia, in the presence of confirmatory electrocardiographic changes without histologic evidence of necrosis, resulted in no significant elevation in the two serum enzyme activities. In most cases, experimental canine pericarditis

and pulmonary infarction were accompanied by no significant rise in SGO-T and SGP-T activity [1,128].

The very high GO-T activity of human cardiac tissue, as opposed to the relatively low activity of an equivalent quantity of serum, results in significant elevation of SGO-T when the enzyme is released from necrotic cardiac tissue following acute myocardial infarction. Other enzymes present in great enough amounts in heart tissue may be expected to behave similarly [170]. Transmural myocardial infarction in the adult man is associated with an elevation in SGO-T which is manifested approximately six to twelve hours after the estimated time of coronary occlusion [28,34,49,77,78,81,90,92,93,113,154,160,170,174]. The rise in serum enzyme activity reaches a peak within twenty-four to forty-eight hours, returning to the normal range by the fourth to the seventh day after infarction. The peak elevations noted following myocardial infarction are two to fifteenfold greater than the normal levels for SGO-T. The peak rise and duration of abnormal serum enzyme activities may be proportional to the size of the infarction and/or the degree of myocardial necrosis. If serum sampling is too infrequent during the first to third day following coronary occlusion, the maximal elevation may be missed. The relationships between the peak SGO-T rise, the duration of abnormal activity, and the size of the infarct are in keeping with the experimental evidence in dogs, as well as with the clinical observation that a poor prognosis is indicated when the SGO-T activity exceeds 300 units at the peak rise after infarction [78,170]. A small myocardial infarction, however, with a minor rise in SGO-T, does not necessarily imply a favorable prognosis.

Following myocardial infarction, the rise in SGO-T activity is not influenced by or correlated with heart failure [101], location of the infarction, administration of digitalis or quinidine, age, sex, color, body temperature, erythrocyte sedimentation rate, leukocyte count or urinary output [90,92,108]. The rise in SGO-T following infarction is not necessarily related to the configuration of the electrocardiogram. When the electrocardiogram is not diagnostic of myocardial infarction or is obscured by previous myocardial infarction, bundle branch block, Wolff-Parkinson-White syndrome and/or other electrocardiographic aberrations, the rise in SGO-T activity in a clinical setting suggestive

of infarction is an especially helpful diagnostic tool [27,60,89,90,170]. Secondary rises in SGO-T activity have been observed in patients who, following acute myocardial infarction, present clinical stigmas consistent with extension of the infarction [27,92].

Changes in SGO-T activity may aid in delineating the process of myocardial infarction in patients with substernal pain, not only with suspected coronary occlusion but also in the presence of coronary insufficiency without occlusion. It appears that the SGO-T activity remains within normal limits in patients with status anginosus or coronary insufficiency in spite of accompanying transient ST segment and T wave abnormalities. When, in these clinical settings of acute coronary insufficiency, the SGO-T activity is increased, the suggestion is that ischemia of cardiac muscle has been accompanied by and/or followed by myocardial necrosis [128,145].

In most but not all instances of pericarditis of various causes, pulmonary emboli with and without pulmonary infarction [60,72,90], cardiac arrhythmias [175] and rheumatic carditis [107,109,129], no elevations (or at most inconsistent elevations) in SGO-T activity have been observed. Following intracardiac surgery, SGO-T activity alterations have been recorded [70,158].

In most instances of acute transmural myocardial infarction and prolonged coronary insufficiency associated with focal myocardial necrosis, the SGP-T activity is not increased [181]. However, when an infarct is sizable, or when hepatic tissue injury due to prolonged anoxia or drug toxicity plays a role, the amount of GP-T liberated is great enough to alter the SGP-T above the normal range.

ALTERATIONS OF SERUM TRANSAMINASE IN HEPATIC DISEASE

Viral hepatitis produced experimentally in mice has been associated with an increase in SGO-T [55] and SGP-T [54] activities. There appears to be a relationship between the degree of elevations in SGO-T activity and the size and virulence of the viral inoculum, the blood virus titer, and the degree of liver necrosis [55]. In mice, the serial alterations of SGO-T in viral hepatitis are paralleled by changes in SGP-T activity, which is increased proportionately to a greater extent above the normal range than is SGO-T activity [42,54]. The injury of hepatic tissue accompanying partial hepatectomy in

mice is associated with elevations in the serum transaminases [55]. The hepatocellular injury resulting from acute toxic hepatitis experimentally produced in rats by administering carbon tetrachloride was shown to be reflected in SGO-T alterations; the amount and duration of increased SGO-T activity was noted to be proportional to the amount of toxin administered and to the extent of liver cell damage [4,179]. Cirrhosis and hepatic tumors, produced experimentally in rats by administration of butter yellow, have been shown to be accompanied by increased SGO-T activity [33,118]. Occlusion of the common duct produced experimentally in dogs resulted in elevations in SGO-T activity which returned to normal within a week following release of the biliary tract obstruction [14,52,146].

Acute hepatic disease in man is associated with increases in SGO-T and SGP-T activity [29,43,179-181,185]. In most instances the quantitative and serial changes in these two serum enzymes are sufficiently characteristic of the various types of liver disease to assist in diagnostic differentiation [56,179,181].

The largest elevations in SGO-T and SGP-T have been observed in acute toxic hepatitis due to carbon tetrachloride, and in patients with acute infectious and/or homologous serum hepatitis [28,43,179,181]. Exposure to carbon tetrachloride results in elevations of both serum enzymes within twenty-four hours, reaching peak levels as high as 27,000 units. With cessation of exposure to the toxin, SGO-T and SGP-T fall precipitously toward normal. The alterations in SGP-T parallel those seen in SGO-T activity, but are usually greater in the latter. Toxic hepatitis due to chlorpromazine [155,177], salicylates [107,175], azaserine [175], pyrazinamide [175], ethyl alcohol [8,2], iproniazid [132], lead [166], bishydroxycoumarin [182], morphine [121], and other agents [53] is associated with elevations in the serum transaminases. Continued increments in the serum enzymes are observed with continued administration of these drugs when they prove to be hepatotoxic; discontinuance of the hepatotoxic agent results in a rapid decrease of the serum transaminases toward normal.

Acute liver cell injury, such as occurs in acute infectious and homologous serum hepatitis, results in impressive increments in the two serum transaminases [5,29,40,43,103,130,171,177,179-181,183]. Although the changes in the activ-

ity of these enzymes parallel each other, the rise of SGP-T usually exceeds that of SGO-T activity. It appears that the rise in serum transaminases in viral and homologous serum hepatitis begins during the prodromal phase of the disease, and reaches a peak elevation which usually is 10 to 100 times greater than the normal when the patients are the sickest, as adjudged by fever, malaise, anorexia, nausea, vomiting and hepatic tenderness. With subjective and objective evidence of improvement, a fall in both serum transaminases toward normal occurs.

The natural, uncomplicated course of infectious hepatitis is associated with a gradual increase in both serum transaminase activities to a peak, followed by a gradual decrease toward the normal range during the recovery phase [171]. When complications occur during the course of hepatitis, this is reflected in a secondary superimposed rise in SGO-T and SGP-T activity. Ambulation during recovery from infectious hepatitis is sometimes associated with a small rise in serum transaminase. If the rise following ambulation is 50 or more units, return to rest is advised, in which case the SGO-T and SGP-T activities usually resume their decline toward normal [171]. Deviations from the usual pattern of serial alterations in SGO-T and SGP-T in the course of acute infectious hepatitis thus suggest associated complications, relapses and/or chronicity of the hepatic infection. It appears that the serial and quantitative changes in SGO-T and SGP-T during the course of hepatitis reflect the clinical state of the patient more accurately than do the usual tests of liver function. In this regard, SGO-T and SGP-T are thought not to reflect liver cell function *per se*, but rather to represent the reaction to acute liver cell injury; accordingly, the serum transaminase alterations are not necessarily correlated with the conventionally employed tests of liver function. Observations during the course of an epidemic of acute infectious hepatitis in a closed environment indicated that elevations in serum transaminase more sensitively reflect subclinical hepatitis than do conventional liver function tests [183].

Infectious mononucleosis is accompanied by normal SGO-T and SGP-T activity unless complicated by hepatitis, in which instance there is a rise in SGO-T and SGP-T [137,181]. The severity of the hepatitis accompanying infectious mononucleosis appears to be related quantitatively to the peak rise in SGO-T and SGP-T,

the latter usually being greater than the former [137].

Active or progressive Laennec's cirrhosis is associated with distinct increases in SGO-T and lesser or no elevations in SGP-T [117,181,186]. The values for SGO-T are in the range of 50 to 250 units [117,179]. Biliary cirrhosis is generally accompanied by somewhat greater elevations than portal cirrhosis [117]. Alterations in SGO-T have proved of little value in distinguishing between primary and secondary biliary cirrhosis [83]. Cirrhosis complicated by acute hepatitis has been shown to exhibit SGO-T and SGP-T elevations quantitatively and serially characteristic of acute hepatitis, superimposed on the serum enzyme elevations identified with hepatic cirrhosis [46,179].

Extrahepatic biliary obstructive jaundice is characterized by increments in transaminase activity from 40 to 300 SGO-T units and 50 to 400 SGP-T units. Although both enzymes are altered in the same direction, in acute extrahepatic biliary obstruction the SGP-T activity usually exceeds the corresponding SGO-T activity [181]. The serum enzyme activities generally return to normal within a week after relief of the biliary obstruction.

Serum glutamic-oxaloacetic transaminase activity has been reported to be as sensitive an index of primary and metastatic cancerous involvement of the liver as the serum alkaline phosphatase, but SGO-T is unaffected by the presence of active metastatic bone cancer [116,178]. The degree of increased SGO-T activity occurring in metastatic cancer to the liver is roughly proportional to the amount of liver cell injury resulting from tumor growth. When SGP-T is increased concomitantly with SGO-T in metastatic liver disease, it is of lower activity than SGO-T [181].

Although a whole battery of liver function blood tests may at times be necessary to help define the etiologic explanation of hyperbilirubinemia in the jaundiced patient, it has recently been suggested that in many instances the laboratory information will suffice if limited to the determination of serum total bilirubin, serum alkaline phosphatase and serum transaminases [59,94,157,173]. Extrahepatic biliary obstructive jaundice is readily differentiated from that due to homologous serum and infectious hepatitis by the fact that the serum alkaline phosphatase is usually higher in the former than in the latter, whereas the

converse is true of SGO-T and SGP-T. As a rule, in the initial or increasing icteric phase of acute hepatitis both serum transaminases are well over 400 units, while in obstructive jaundice the serum transaminases are usually below 400. In both instances, SGP-T activity is greater than the simultaneously measured SGO-T activity. In addition, the serial alterations in the serum enzymes in obstructive jaundice and in jaundice associated with acute hepatitis are readily distinguishable, being quantitatively distinct. Toxic hepatitis associated with the ingestion of drugs may mimic the serum enzyme alterations seen in obstructive jaundice, as when the serum alkaline phosphatase is appreciably elevated, but hepatitis due to hepatotoxic agents may be distinguished from obstructive jaundice and acute hepatitis by means of serum transaminase determinations. When the insult to the liver is stopped by the discontinuance of hepatotoxic agents, the serum transaminases begin to fall toward normal even though the serum bilirubin and/or serum alkaline phosphatase may remain unchanged or even increase transiently.

When acute hepatitis is superimposed on a liver containing metastatic deposits the SGO-T activity may be greater than the SGP-T activity; this turn of events has been found to be associated with an abnormal serum protein electrophoretic pattern, including elevated β - and γ -globulins. However, the values of both serum transaminases are usually greater than 400 units in the initial or increasing icteric phase of the hepatitis. When obstructive jaundice occurs in a patient with metastatic liver disease, SGP-T activity may be greater than, equal to, or somewhat less than the SGO-T activity. The serum transaminase values rarely exceed 300 units, even when the serum bilirubin is 30 mg. per cent or more.

In neonatal jaundice, determination of the serum transaminases may be of help in the differential diagnosis of jaundice [86-88].

ALTERATIONS IN SERUM TRANSAMINASE IN PATHOLOGIC SKELETAL MUSCLE STATES

Trauma to skeletal muscle during the course of experimental surgical procedures on animals is accompanied by a moderate increase in SGO-T activity and a slight increase in SGP-T activity [97]. Surgical trauma in human subjects also has been observed to result at times in elevations of serum transaminase [11,36,38,125,134]. There appears to be a relationship between

the degree of muscle trauma and the peak rise in SGO-T [97,100]. The SGO-T activity cannot be used as a specific criterion of cardiac injury in accident victims, inasmuch as persons experiencing extensive trauma may show SGO-T activity increments unrelated to demonstrable cardiac injury [100].

In a study of neuromuscular diseases, SGO-T was found to be elevated in progressive muscular dystrophy [122], pseudohypertrophic muscular dystrophy [138], dermatomyositis [41,92,156], and in gangrene of the toes. Amyotrophic lateral sclerosis, progressive muscular atrophy, myasthenia gravis, rheumatoid musculoskeletal diseases and nerve section [9] were not associated with increased serum transaminase activity.

ALTERATIONS IN SERUM TRANSAMINASE IN OTHER ABNORMAL STATES

Azotemia and uremia associated with acute and chronic renal disease have not been found to be associated with elevations in SGO-T [28,92,178]. No extensive experience with renal infarcts has been reported. In the production of experimental graded renal infarcts in dogs by arterial ligation [135,142], it has been observed that SGO-T activity is increased above the normal range in proportion to the size of the infarct.

Acute pancreatitis [29] is inconstantly associated with rises in SGO-T and SGP-T. Whether these alterations are reflections of release of enzyme from necrotic and/or inflammatory pancreatic tissue, or are due to transient biliary obstruction caused by edema around the extrahepatic biliary ducts is not clear.

Although injury to central nervous tissue [156] is usually unaccompanied by significant alterations in SGO-T and SGP-T, or by transient increases at most [108], elevations of SGO-T have been observed in instances of extensive cerebral tissue injury associated with massive cerebral hemorrhages and thromboses [101]. The absence of intracellular enzymes from the blood stream after central nervous tissue injury is presumed to reflect the influence of a blood-brain barrier [39,51,63,64,102].

Experimental studies in dogs revealed a relationship between experimental cerebral infarction and the GO-T activity of cerebrospinal fluid [165]. Studies [39,51,61,63,64,79,124,153] indicate that increased activity of GO-T of cerebrospinal fluid may occur in a number of

neurologic diseases. In most of the diseases studied, no correlation between the activity of transaminase and the protein content of the cerebrospinal fluid was observed [39,51,64]. In comparison with the findings in cerebral infarction in man, infarctions produced experimentally in dogs led to considerably greater and more consistent increases in cerebrospinal fluid transaminase [51,165].

The normal range of GO-T activity of cerebrospinal fluid obtained from persons without disease of the central nervous system has differed in various reports [39,51,64]. These differences contribute to the divergent interpretations of the changes of enzyme activity observed in pathologic states of the central nervous system.

Reports on the clinical significance of alterations in cerebrospinal fluid transaminase activity also differ. Increases in GO-T activity in cerebrospinal fluid usually are correlated with acute and significant injury in the central nervous system, of diverse causes including those of thromboembolic, degenerative, infectious and neoplastic origin. The increase in transaminase activity appears to occur at varying times after onset of the central nervous tissue injury. However, clinically significant central nervous tissue injury may occur without increased cerebrospinal fluid transaminase activity. No correlation has been found between the serum transaminase activity and the enzyme activity of the cerebrospinal fluid [39,64], nor has any relationship been observed between the transaminase activity and the leukocyte or erythrocyte count, protein, glucose or chloride content, or any other laboratory parameters of alteration in the cerebrospinal fluid. From the data presently available it would appear that the lack of specificity and the relative insensitivity of changes in cerebrospinal fluid transaminase activity limit the clinical usefulness of this enzyme as a reflection of disease of the central nervous system [39].

In the absence of cardiac and hepatic disease, SGO-T and SGP-T activities are normal in pregnancy with the exception of the toxemias [12,28,37,84,108,123].

Reports of serum transaminase activity alterations in hematopoietic diseases have been inconclusive [104,148,163,168]. Radiation has a varying influence on SGO-T activity [18,26,82,114]. Elevations of SGO-T activity have been observed following positive radial acceleratory force [184].

TABLE I
RANGE OF SGO-T AND SGP-T VALUES IN DISEASE STATES

| Disease States | Range of SGO-T Activity* | Range of SGP-T Activity* |
|--|--------------------------|--------------------------|
| Normal range | 8-40 | 5-35 |
| Transmural myocardial infarction | 50-600 | 5-150 |
| Subendocardial infarction | 20-150 | 5-50 |
| Viral and/or homologous serum hepatitis | | |
| Non-icteric phase | 50-300 | 60-400 |
| Increasing icteric phase | 500-2500 | 600-3500 |
| Toxic hepatitis | 50-27,000 | 50-20,000 |
| Laennec's cirrhosis, progressive | 50-250 | 30-200 |
| Biliary cirrhosis, progressive | 50-350 | 30-300 |
| Extrahepatic biliary tract obstruction | 40-300 | 50-400 |
| Metastatic and primary hepatic carcinoma | 40-250 | 20-150 |
| Skeletal muscle trauma | 30-500 | 20-150 |
| Progressive muscular dystrophy | 40-250 | 20-100 |
| Pseudohypertrophic muscular dystrophy | | |
| Dermatomyositis | | |

* Spectrophotometric units.

CONCLUSIONS

The glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase activities of the serum are readily measurable by relatively simple technics.

Diagnostically significant alterations of these serum enzymes have been observed, notably during the course of cardiac, hepatic and muscular diseases. These reflect enzyme changes at the intracellular level of the respective tissues. (Table I.)

Although a multiplicity of diseases are associated with increased serum transaminases these are of diagnostic aid when correlated with the clinical facts.

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REFERENCES

1. AGRESS, C. M., GLASSNER, H. F. and JACOBS, J. I. Serum transaminase levels in experimental pulmonary infarction. *Circulation Res.*, 4: 220-222, 1956.
2. ALLGÉN, S. I., NAUCKHOFF, B., ORDELL, I., SALUM, I. and LZIKOWITZ, S. Serum diphosphopyridine

- nucleotide-linked enzymes in delirium tremens and allied conditions. *Science*, 128: 304-305, 1958.
3. AMES, S. R., SARMA, P. S. and ELVEHJEM, C. A. Transaminase and pyridoxine deficiency. *J. Biol. Chem.*, 167: 135-141, 1947.
4. ASADA, M. Transaminase activity in liver damage. i. Study on experimental liver damage. *M. J. Osaka Univ.*, 9: 45-55, 1958.
5. ASADA, M. Transaminase activity in liver damage. ii. Study on various liver diseases. *M. J. Osaka Univ.*, 9: 57-71, 1958.
6. AWAPARA, J. and SEALE, B. Distribution of transaminase in rat organs. *J. Biol. Chem.*, 194: 497-502, 1952.
7. BABCOCK, M. J. Serum glutamic-oxaloacetic transaminase activity of vitamin B₆-deficient rats. *J. Nutrition*, 67: 205-212, 1959.
8. BANG, N. U. Serum glutamic oxaloacetic transaminase activity in acute and chronic alcoholism. *J. A. M. A.*, 168: 156-160, 1958.
9. BARR, J. H., JR. Serum glutamic oxaloacetic transaminase in rheumatoid arthritis and certain rheumatoid musculoskeletal disorders. *Arthritis & Rheumatism*, 1: 147-150, 1958.
10. BJÖRCK, G. and HANSON, A. Glutamic-oxaloacetic transaminase in the diagnosis of myocardial infarction. *Acta med. Scandinav.*, 155: 317-320, 1956.
11. BONATI, B., LACERENZ, C., RANCATI, G. B. and TENCONI, L. T. Comparative studies on the behavior of a hematic and hepatic glutamic-oxaloacetic transaminase in various pathological conditions in surgical shock. *Minerva med.*, 47: 267-272, 1956.
12. BOWEN, J., WRÓBLEWSKI, F. and LADUE, J. S. Unpublished observations.
13. BOWERS, G. N., JR. An accurate simplification of the serum glutamic-oxaloacetic and pyruvic transaminases—method, observations and normal values. *Am. J. Clin. Path.*, 30: 1-6, 1958.
14. BRAUN, P., PAPP, M. and HORVATH, I. Determination of transaminase activity in dogs' serum and lymph after hepatic damage due to acute biliary obstruction. *Nature, London*, 183: 48-49, 1959.
15. BRAUNSHTEIN, A. E. Transamination and the integrative functions of the dicarboxylic acids in nitrogen metabolism. *Advances Prot. Chem.*, 3: 152, 1947.
16. BRAUNSHTEIN, A. E. and KRITSMAN, M. G. Formation and breakdown of amino acids by intermolecular transfer of amino groups. *Nature, London*, 140: 503-504, 1937.
17. BRAUNSHTEIN, A. E. and KRITSMAN, M. G. Über den Ab- und Aufbau von Aminosäuren durch Umaminierung. *Enzymologia*, 2: 129-146, 1937.
18. BRENT, R. L., McLAUGHLIN, M. M. and STABILE, J. N. The effect of irradiation on the serum glutamic-oxaloacetic transaminase level. *Radiation Res.*, 9: 24-32, 1958.
19. BRUCE, R., TODD, J. K. and LEDUNE, L. Serum transaminase: its clinical use in diagnosis and prognosis. *Brit. M. J.*, 2: 1125-1128, 1958.
20. BRUYET, P., MEIGNIEN-GOUTHIER, C. and DELAUNAY, A. Serum transaminases and their clinical interest. ii. Various affections. *Presse Méd.*, 66: 93, 2131, 1958.

21. BURSTEIN, J. and HARJANNE, A. Glutamic-oxaloacetic transaminase and C-reactive protein in myocardial infarction, with special reference to the electrocardiographic pattern. *Acta med. Scandinav.*, 163: 175-184, 1959.
22. CABAUD, P., LEEPER, R. and WRÓBLEWSKI, F. Colorimetric measurement of serum glutamic-oxaloacetic transaminase. *Am. J. Clin. Path.*, 26: 1101-1105, 1956.
23. CAMMARATA, P. S. and COHEN, P. P. Fractionation and properties of glutamic-oxaloacetic transaminase. *J. Biol. Chem.*, 193: 53-62, 1951.
24. CAMMARATA, P. S. and COHEN, P. P. Unpublished observations.
25. CAMMARATA, P. S. and COHEN, P. P. The scope of the transamination reaction in animal tissues. *J. Biol. Chem.*, 187: 439-452, 1950.
26. CANDURA, F. and TIMOSI, G. Behavior of blood glutamic-oxaloacetic transaminase in patients exposed to x-ray therapy. *Progr. med. Nap.*, 13: 265-270, 1957.
27. CHINSKY, M. and SHERRY, S. Serum transaminase as a diagnostic aid. *Arch. Int. Med.*, 99: 556-558, 1957.
28. CHINSKY, M., SHMAGRANOFF, G. L. and SHERRY, S. Serum transaminase activity: observations in a large group of patients. *J. Lab. & Clin. Med.*, 47: 108-118, 1956.
29. CHINSKY, M., WOLFF, R. J. and SHERRY, S. Serum transaminase activity: a comparison of the pyruvic and oxaloacetic transaminases. *Am. J. M. Sc.*, 233: 400-408, 1957.
30. COHEN, P. P. Kinetics of transaminase activity. *J. Biol. Chem.*, 136: 585-601, 1940.
31. COHEN, P. P. Transaminases. In: *The Enzymes*, vol. 1, Pt. 2, pp. 1040-1067. Edited by Sumner, J. B. and Myrback, K. New York, 1951. Academic Press.
32. COHEN, P. P., and HEKHIUS, G. L. Rate of transamination in normal tissues. *J. Biol. Chem.*, 140: 711-724, 1941.
33. COHEN, P. P., HEKHIUS, G. L. and SOBER, M. S. Transamination in liver from rats fed butter yellow. *Cancer Res.*, 2: 405-410, 1942.
34. CONRAD, F. G. Transaminase. *New England J. Med.*, 256: 602-608, 1957.
35. CORNELIUS, C. E., BISHOP, J., SWITZER, J. and RHODE, E. A. Serum and tissue transaminase activities in domestic animals. *Cornell Vet.*, 49: 116-126, 1959.
36. CRAVER, W. L., JOHNSON, G., JR. and BEAL, J. M. Alterations in serum glutamic-oxaloacetic transaminase activity following operations. *S. Forum*, 8: 77-80, 1957.
37. CRISP, W. E., MIESFELD, R. L. and FRAJOLA, W. J. Serum glutamic oxaloacetic transaminase levels in the toxemias of pregnancy. *Obst. & Gynec.*, 13: 487-597, 1959.
38. D'ABBICCO, V. and TULLIO, A. D. Variation of the serum aminopherase (transaminase) activities in surgical patients. *J. A. M. A.*, 169: 77-146, 1959.
39. DECKER, B. J., CARDELLO, L. and WRÓBLEWSKI, F. The clinical significance of cerebrospinal fluid transaminase. Unpublished observations.
40. DELKESKAMP, A., SCHMIDT, E. and SCHMIDT, F. A. Measurement of enzyme activity in the diagnosis of anicteric hepatitis. *German M. Monthly*, 4: 44-46, 1959.
41. DE MORAGAS, J. M., PERRY, H. O. and FLEISHER, G. A. Serum glutamic-oxaloacetic transaminase in dermatomyositis. *J. A. M. A.*, 166: 1936-1938, 1957.
42. DERITIS, F., COLTORTI, M. and GIUSTI, G. Serum and liver transaminase activities in experimental virus hepatitis in mice. *Science*, 124: 32, 1956.
43. DERITIS, F., COLTORTI, M. and GIUSTI, G. Diagnostic value and pathogenic significance of transaminase activity changes in viral hepatitis. *Minerva med.*, 47: 161-171, 1956.
44. DE TORREGROSA, M. V. Clinical evaluation of a simple colorimetric method for transaminase determination. *Bol. Asoc. méd. Puerto Rico*, 50: 45-55, 1958.
45. DEWAR, H. A., ROWELL, N. R. and SMITH, A. J. Serum glutamic-oxaloacetic transaminase in acute myocardial infarction. *Brit. M. J.*, 2: 1121-1125, 1958.
46. DONATO, R. A. Transaminase activity and morphologic alterations in human livers. *Am. J. Clin. Path.*, 28: 377-384, 1957.
47. DUBACH, U. C. Diagnostic value of glutamic-oxaloacetic acid transaminase. *Ztschr. klin. Med.*, 154: 593-622, 1957.
48. DUNN, M., MARTINS, J. and REISSMANN, K. R. The disappearance rate of glutamic-oxaloacetic transaminase from the circulation and its distribution in the body's fluid compartments and secretions. *J. Lab. & Clin. Med.*, 51: 259-265, 1958.
49. Editorial. Transaminase in myocardial infarction. *Brit. M. J.*, 1: 573, 1957.
50. FALES, F. W. A simplified procedure for the determination of serum transaminase. *Clin. Chem.*, 2: 249, 1956.
51. FLEISHER, G. A., WAKIM, K. G. and GOLDSTEIN, N. P. Glutamic-oxaloacetic transaminase and lactic dehydrogenase in serum and cerebrospinal fluid of patients with neurological disorders. *Proc. Staff Meet., Mayo Clin.*, 32: 188-197, 1957.
52. FORTNER, J. and WRÓBLEWSKI, F. Unpublished observations.
53. FRANKL, H. D. Toxic vapor inhalation and serum enzyme levels. *Texas Rep. Biol. & Med.*, 15: 868-873, 1957.
54. FRIEND, C. and WRÓBLEWSKI, F. Unpublished observations.
55. FRIEND, G., WRÓBLEWSKI, F. and LADUE, J. S. Glutamic-oxaloacetic transaminase activity of serum in mice with viral hepatitis. *J. Exper. Med.*, 102: 699-704, 1955.
56. FURUKAWA, K. and OKUMURA, M. A clinical study on transaminase: simultaneous measurement of serum glutamic-oxaloacetic and glutamic-pyruvic transaminase activities in hepatic and some other disorders. *Kyushu J. M. Sc.*, 9: 38-45, 1958.
57. GLASSNER, H. F., AGRESS, C. M., JACOBS, H. I., LEDERER, M., CLARK, W. G., WRÓBLEWSKI, F. and LADUE, J. S. Serum transaminase levels in experimental myocardial infarction. *Am. J. Physiol.*, 179: 639, 1954.
58. GOBLE, A. J. and O'BRIEN, E. N. Acute myocardial

- ischemia: significance of plasma-transaminase activity. *Lancet*, 2: 873-877, 1958.
59. GOLDSTEIN, F., SELIGSON, D. and BOCKUS, H. L. Serum glutamic-oxaloacetic transaminase and iron in the differential diagnosis of jaundice. *Gastroenterology*, 36: 487-500, 1959.
 60. GOLDSTEIN, F., ISRAEL, H. L. and SELIGSON, D. Use of serum transaminase levels in the differentiation of pulmonary embolism and myocardial infarction. *New England J. Med.*, 254: 746-749, 1956.
 61. GREEN, D. E., LELOIR, L. F. and NOCITO, V. J. Transaminase. *J. Biol. Chem.*, 161: 559-582, 1945.
 62. GREEN, J. B. Cerebrospinal-fluid transaminase concentrations in clinical cerebral infarctions. *New England J. Med.*, 256: 220-221, 1957.
 63. GREEN, J. B., O'DOHERTY, D. S., OLDEWURTEL, H. A. and FORSTER, F. M. Cerebrospinal fluid transaminase in clinical cerebral infarction: a preliminary report. *New England J. Med.*, 256: 220-221, 1957.
 64. GREEN, J. B., OLDEWURTEL, H. A., O'DOHERTY, D. S., FORSTER, F. M. and SANCHEZ-LONGO, L. P. Cerebrospinal fluid glutamic-oxaloacetic transaminase activity in neurologic diseases. *Neurology*, 7: 313-322, 1957.
 65. GROEN, A., SAUVEPLANNE, A. L. and KOOPS, G. Experiences with Frankel's and Reitman's blood glutamic-oxaloacetic transaminase determination with special reference to myocardial infarct. *Nederl. tijdschr. geneesk.*, 102: 1641-1645, 1958.
 66. HANSEN, P. F. and LAURSEN, T. Serum glutamic-oxaloacetic transaminase in myocardial infarction. Its diagnostic and prognostic value. *Danish M. Bull.*, 4: 248-251, 1957.
 67. HENLEY, K. S. and POLLARD, H. M. A new method for the determination of glutamic-oxaloacetic and glutamic-pyruvic transaminase in plasma. *J. Lab. & Clin. Med.*, 46: 785-789, 1955.
 68. HERGT, K. and LANGIN, J. L. Serum transaminase determination: a simplified adaptation to the Beckman model B spectrophotometer and some clinical applications in the general hospital. *Am. J. M. Sc.*, 233: 69-76, 1957.
 69. HERMANN, F., BROOKS, M. and STORVICK, C. Micro determination of glutamic-oxaloacetic transaminase in blood serum. *Clin. Chem.*, 5: 57-61, 1959.
 70. HOWARD, H. S. and NEELY, W. A. Serum transaminase following ventriculotomy. *Am. J. Med.*, 25: 124, 1958.
 71. JENKINS, W. T., YPHANTIS, D. A. and SIZER, I. W. Glutamic aspartic transaminase. I. Assay, purification, and general properties. *J. Biol. Chem.*, 234: 51-57, 1959.
 72. KALMANSOHN, R. B. and KALMANSOHN, R. W. An evaluation of the serum glutamic-oxaloacetic transaminase activity in pericarditis. *Am. Heart J.*, 55: 739-742, 1958.
 73. KALTENBACH, J. P., BECKER, J. F. and BERNSTEIN, I. Simplified spectrophotometric assay of serum glutamic-oxaloacetic transaminase and lactic dehydrogenase. *Am. J. Clin. Path.*, 27: 309-314, 1957.
 74. KARMEN, A. A note in the spectrophotometric assay of glutamic-oxaloacetic transaminase in human blood serum. *J. Clin. Invest.*, 34: 131-133, 1955.
 75. KARMEN, A., WRÓBLEWSKI, F. and LADUE, J. S. Quantitative estimation of glutamic-oxaloacetic transaminase activity in human serum. *Clin. Res. Proc.*, 1: 90, 1953.
 76. KARMEN, A., WRÓBLEWSKI, F. and LADUE, J. S. Transaminase activity in human blood. *J. Clin. Invest.*, 34: 126-133, 1955.
 77. KATTUS, A. A., JR., WATANABE, R. and SEMENSON, C. Diagnostic and prognostic significance of serum transaminase levels in coronary occlusive disease. *Circulation*, 15: 502-511, 1957.
 78. KATTUS, A. A., JR., WATANABE, R., SEMENSON, C., DRELL, W. and AGRESS, C. M. Serum aminophosphatase (transaminase) in the diagnosis of acute myocardial infarction. *J. A. M. A.*, 160: 16-20, 1956.
 79. KATZMAN, R., FISHMAN, R. A. and GOLDENSOHN, E. S. Glutamic-oxaloacetic transaminase activity in spinal fluid. *Neurology*, 7: 853-855, 1957.
 80. KEELE, K. D., GOULDEN, F. and NEUMAN, M. J. Prognostic and diagnostic value of serum glutamic-oxaloacetic transaminase in suspected cardiac infarction. *Lancet*, 2: 1187-1189, 1958.
 81. KESSLER, G. and PHELPS, A. Serum glutamic-oxaloacetic transaminase. *J. Albert Einstein M. Center*, 4: 91-94, 1956.
 82. KESSLER, G., HERMEL, M. B. and GERSHON-COHEN, J. Serum glutamic-oxaloacetic transaminase activity after whole body irradiation. *Proc. Soc. Exper. Biol. & Med.*, 98: 201-203, 1958.
 83. KLECKNER, M. S. Determination of iron, mucoprotein, transaminase and cholinesterase in the serum in the differentiation of primary biliary cirrhosis, secondary biliary cirrhosis and cholestatic hepatic disease. *Clin. Res. Proc.*, 5: 211, 1957.
 84. KNUTSON, R. G., CORNATZER, W. E., MOORE, J. H. and NELSON, W. W. Serum lactic dehydrogenase and glutamic-oxaloacetic transaminase activities in normal pregnancy. *J. Lab. & Clin. Med.*, 51: 773-777, 1958.
 85. KOCHAKIAN, C. D. and ENDAHL, B. R. Effect of homogenizing medium and nutritive state on transaminase activities of tissues. *Proc. Soc. Exper. Biol. Med.*, 98: 284-285, 1958.
 86. KOVE, S., GOLDSTEIN, S. and WRÓBLEWSKI, F. Activity of glutamic-oxaloacetic transaminase in the serum in the neonatal period. *Pediatrics*, 20: 584-589, 1957.
 87. KOVE, S., GOLDSTEIN, S. and WRÓBLEWSKI, F. Measurement of activity of transaminases in the serum as an aid in the diagnosis of jaundice in the neonatal period. *Pediatrics*, 20: 590-600, 1957.
 88. KOVE, S., GOLDSTEIN, S. and WRÓBLEWSKI, F. Serum transaminase activity in neonatal period: valuable aid in differential diagnosis in jaundice of the newborn infant. *J. A. M. A.*, 168: 860-867, 1958.
 89. KRAUSE, S. and KRAUSE, G. Serum glutamic-oxaloacetic aminophosphatase (transaminase) determinations: value in the diagnosis of acute myocardial infarction in the presence of left bundle-branch block. *J. A. M. A.*, 161: 144-147, 1956.
 90. KRAUSE, S., HECKERT, W. E., SOLINAS, P. and BOGARAD, I. M. The value of serum aminophosphatase determinations in suspected acute myocardial infarction. *Dis. Chest*, 31: 512-524, 1957.

91. LADUE, J. S. and WRÓBLEWSKI, F. Serum glutamic-oxaloacetic transaminase variations in heart and liver disease. In: A. M. A. Scientific Exhibits, pp. 297-304. New York, 1955. Grune & Stratton.
92. LADUE, J. S. and WRÓBLEWSKI, F. The significance of the serum glutamic-oxaloacetic transaminase activity following acute myocardial infarction. *Circulation*, 11: 871-877, 1955.
93. LADUE, J. S., WRÓBLEWSKI, F. and KARMEN, A. Serum glutamic-oxaloacetic transaminase activity in human acute transmural myocardial infarction. *Science*, 120: 497-499, 1954.
94. LATNER, A. L. and SMITH, A. J. Serum-transaminase alkaline-phosphatase ratio in differential diagnosis of jaundice. *Lancet*, 2: 915-917, 1958.
95. LAURSEN, T. and ESPERSEN, G. A fluorimetric method for measuring the activity in serum of the enzyme glutamic oxaloacetic transaminase. *Scandinav. J. Lab. & Clin. Invest.*, 11: 61-65, 1959.
96. LAURSEN, T. and HANSEN, P. A fluorimetric method for measuring the activity in serum of the enzyme glutamic-pyruvic transaminase. *Scandinav. J. Lab. & Clin. Invest.*, 10: 53-58, 1958.
97. LEMLEY-STONE, J. M., MERRILL, J. M., GRACE, J. T. and MENEELY, G. R. Transaminase in experimental myocardial infarction. *Am. J. Physiol.*, 182: 555-558, 1955.
98. LENARD, P. and STRAUB, F. B. Aminopherase. *Stud. Inst. Med. Chem. Univ. Szeged.*, 2: 59-71, 1942.
99. LICHSTEIN, H. C., GUNSALUS, I. C. and UMBREIT, W. W. Function of vitamin B₆ group; pyridoxal phosphate (co-decarboxylase) in transamination. *J. Biol. Chem.*, 161: 311-320, 1945.
100. LIEBERMAN, J., LASKY, I. I., DULKIN, S. I. and LOBSTEIN, O. E. Serum glutamic-oxaloacetic transaminase activity in conditions associated with myocardial infarction. i. Body of trauma. *Ann. Int. Med.*, 46: 485-496, 1957.
101. LIEBERMAN, J., LASKY, I. I., DULKIN, S. I. and LOBSTEIN, O. E. Serum glutamic-oxaloacetic transaminase activity in conditions associated with myocardial infarction. ii. Cerebral vascular accidents and congestive heart failure. *Ann. Int. Med.*, 46: 497-505, 1957.
102. LIEBERMAN, J., DAIBER, O., DULKIN, S. I., LOBSTEIN, O. E. and KAPLAN, M. R. Glutamic-oxaloacetic transaminase in serum and cerebrospinal fluid of patients with cerebrovascular accidents: demonstration of a blood-cerebrospinal fluid barrier. *New England J. Med.*, 257: 1201-1207, 1957.
103. MADSEN, S., BANG, N. U. and IVERSEN, K. Serum glutamic-oxaloacetic transaminase in diseases of the liver and biliary tract. *Brit. M. J.*, 1: 543-546, 1958.
104. MAGALINE, S. I. and STEFANINI, M. Studies on platelets. xvi. Glutamic-oxaloacetic transaminase activity in human platelets. *Proc. Soc. Exper. Biol. & Med.*, 91: 404-406, 1956.
105. MAGILL, G. B., WRÓBLEWSKI, F. and LADUE, J. S. Serum lactic dehydrogenase and serum transaminase in human leukemia. *Blood*, 14: 870-881, 1959.
106. MANSO, C. and WRÓBLEWSKI, F. Reacoes de transaminacao no sangue humano. *Med. Contemporanea.*, 75: 7-12, 1957.
107. MANSO, C., TARANTA, A. and NYDICK, I. Effect of aspirin on serum glutamic-oxaloacetic and serum pyruvic-transaminase in children. *Proc. Soc. Exper. Biol. & Med.*, 93: 84-88, 1956.
108. MASON, J. H. and WRÓBLEWSKI, F. Serum glutamic-oxaloacetic transaminase activity in experimental and disease states. *Arch. Int. Med.*, 99: 245-252, 1957.
109. MASSIE, R. W. and STAHLMAN, M. Serum oxaloacetic transaminase activity in acute rheumatic fever. *J. Dis. Child.*, 95: 469-476, 1958.
110. MEISTER, A. Transamination. *Advances Enzymol.*, 16: 185-246, 1955.
111. MEISTER, A., SOBER, H. A. and PETERSON, E. A. Activation of purified glutamic-aspartic apotransaminase by crystalline pyridoxamine phosphate. *J. Am. Chem. Soc.*, 74: 2385-2386, 1952.
112. MERRILL, J. M., STONE, J. L., GRACE, J. T. and MENEELY, G. R. Decreased activity of glutamic-oxaloacetic transaminase in infarcted myocardial tissues of the dog. *Clin. Res. Proc.*, 3: 113, 1955.
113. MERRILL, J. M., STONE, J. L., GRACE, J. T. and MENEELY, G. R. Recent clinical experiences with serum aminopherase (transaminase) determinations. *J. A. M. A.*, 160: 1454-1456, 1956.
114. MILCH, L. J. and ALBAUM, H. G. Serum transaminase activity in x-irradiated rabbits. *Proc. Soc. Exper. Biol. & Med.*, 93: 595-596, 1956.
115. MOHUN, A. F. and COOK, I. J. Y. Simple methods for measuring serum levels of the glutamic-oxaloacetic and glutamic-pyruvic transaminases in routine laboratories. *J. Clin. Path.*, 10: 394-399, 1957.
116. MOLANDER, D. W. Comparative value of transaminase, cholinesterase and alkaline phosphatase in primary and metastatic neoplasms of the liver. *Gastroenterology*, 34: 1047, 1958.
117. MOLANDER, D. W., SHEPPARD, E. and PAYNE, M. A. Serum transaminase in liver disease. *J. A. M. A.*, 163: 1461-1465, 1957.
118. MOLANDER, D. W., WRÓBLEWSKI, F., and LADUE, J. S. A study of serum transaminase in rats with liver dysfunction. *Clin. Res. Proc.*, 3: 48-49, 1955.
119. MOLANDER, D. W., WRÓBLEWSKI, F. and LADUE, J. S. Serum glutamic-oxaloacetic transaminase as an index of hepatocellular integrity. *J. Lab. & Clin. Med.*, 46: 831-839, 1955.
120. MOORE, C. B., BIRCHALL, R., HORACK, H. M. and BATSON, H. M. Changes in serum glutamic oxaloacetic transaminase in patients with diseases of the heart, liver, or musculoskeletal systems. *Am. J. M. Sc.*, 234: 528-537, 1957.
121. MOSSBERG, S. M., BLOOM, A., BERKOWITZ, J. and ROSS, G. Effect of morphine on the serum glutamic-oxaloacetic transaminase (SGO-T) and serum alkaline phosphatase (SAP) activities of normal persons and those with gallbladder disease. *Circulation*, 18: 761-762, 1958.
122. MURPHY, E. G. and CHERNIAK, M. M. Glutamic oxaloacetic transaminase activity in the serum in muscular dystrophy and other neuromuscular disorders in childhood. *Pediatrics*, 22: 1110-1114, 1958.
123. MURPHY, L., WRÓBLEWSKI, F. and LADUE, J. S. Unpublished observations.
124. MYERSON, R. M., HURWITZ, J. K. and SALL, T. Serum and cerebrospinal-fluid transaminase

- concentrations in various neurologic disorders. *New England J. Med.*, 257: 273-276, 1957.
125. NICKELL, W. K. and ALLBRITTEN, F. F. Serum transaminase content related to tissue injury. *Surgery*, 42: 240-248, 1957.
 126. NISONOFF, A., HENRY, S. S. and BARNES, F. W., JR. Mechanisms in enzymatic transamination: variables in the spectrophotometric estimation of glutamic-aspartate kinetics. *J. Biol. Chem.*, 199: 699-711, 1952.
 127. NYDICK, I., WRÓBLEWSKI, F. and LADUE, J. S. Evidence for increased serum glutamic-oxaloacetic transaminase (SGO-T) activity following graded myocardial infarcts in dogs. *Circulation*, 12: 161-168, 1955.
 128. NYDICK, I., RUEGSEGG, P., WRÓBLEWSKI, F. and LADUE, J. S. Variations in serum glutamic-oxaloacetic transaminase activity in experimental and clinical coronary insufficiency, pericarditis and pulmonary infarction. *Circulation*, 15: 324-334, 1957.
 129. NYDICK, I., TANG, J., STOLLEMAN, G. H., WRÓBLEWSKI, F. and LADUE, J. S. A study of changes in serum concentrations of the enzyme, glutamic-oxaloacetic transaminase in rheumatic fever. *Circulation*, 12: 754, 1955.
 130. O'BRIEN, E. N. Plasma-transaminase activity as an index of the effectiveness of cortisone in chronic hepatitis. *Lancet*, 1: 1245-1249, 1958.
 131. O'KANE, D. E. and GUNSALUS, I. C. The resolution and purification of glutamic-aspartic transaminase. *J. Biol. Chem.*, 170: 425-439, 1947.
 132. PARE, C. M. B. and SANDLER, M. Acute hepatic necrosis following iproniazid therapy. Value of glutamic-oxaloacetic transaminase estimation in early detection. *Lancet*, 1: 282-284, 1959.
 133. PEARCE, J. M. and PREUSS, J. W. Serum transaminase changes in rabbits with cardiac lesions produced by virus. III. *Lab. Invest.*, 5: 145-154, 1956.
 134. PERSON, D. A. and JUDGE, R. D. Effect of operation on serum transaminase levels. *Arch. Surg.*, 77: 892-897, 1958.
 135. PRICE, J. V., WRÓBLEWSKI, F. and LADUE, J. S. Unpublished observations.
 136. REITMAN, S. and FRANKEL, S. A colorimetric method for the determination of serum glutamic-oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Path.*, 28: 56-63, 1957.
 137. RENNIE, I. and WRÓBLEWSKI, F. The clinical significance of serum transaminase in infectious mononucleosis hepatitis. *New England J. Med.*, 257: 547-553, 1957.
 138. RITTER, J. A. and SELIGSON, D. Elevations of serum glutamic-oxaloacetic transaminase and urinary corpporphyrins in pseudohypertrophic muscular dystrophy. *Am. J. M. Sc.*, 233: 559-562, 1957.
 139. ROSALKI, S. B. and WILKINSON, J. H. A source of error in serum glutamic oxaloacetic transaminase determination. *J. Clin. Path.*, 12: 138-139, 1959.
 140. RUBINO, F. and MONACO, P. Glutamic oxaloacetic transaminase activity in the heart, diaphragm, and skeletal muscles of normal and fasting guinea pigs. *Boll. Soc. ital. biol. sper.*, 34: 154-156, 1958.
 141. RUDOLPH, K. A. Serum glutamic-oxaloacetic transaminase in experimental tissue injury. *J. Lab. & Clin. Med.*, 49: 31-40, 1957.
 142. RUDOLPH, L. A., DUTTON, R. E., JR. and SHAEFER, J. A. Glutamic-oxaloacetic transaminase levels in experimental tissue damage. *J. Clin. Investigation*, 34: 1960, 1955.
 143. RUDOLPH, L. A., DUTTON, R. E., JR., LINDEMAN, R. and LYONS, R. H. Serum glutamic-oxaloacetic transaminase in tissue ischemia. *Clin. Res. Proc.*, 4: 102, 1956.
 144. RUEGSEGG, P., NYDICK, I., FREIMAN, A. and LADUE, J. S. Serum activity patterns of glutamic-oxaloacetic transaminase, glutamic pyruvic transaminase and lactic dehydrogenase following graded myocardial infarction in dogs. *Circulation Res.*, 7: 4-10, 1959.
 145. RUSKIN, A., MAHAFFEY, W. C. and RUSKIN, B. Serum glutamic-oxaloacetic transaminase. *Clin. Res. Proc.*, 4: 30, 1956.
 146. RUSKIN, A., PATTERSON, M., SINCLAIR, J., WOLMA, F., HAASIS, L. and RUSKIN, B. Alterations in liver and serum enzyme activities under conditions of portal venous ischemia, hepatic venous congestion and common bile duct ligation. *Clin. Res. Proc.*, 7: 154, 1959.
 147. SAMPSON, J. J. Serum transaminase and other enzymes in acute myocardial infarction. *Progr. Cardiovascular Dis.*, 1: 187-205, 1958.
 148. SASS, M. and SPEAR, P. W. Whole blood transaminase levels in anemia. *J. Lab. & Clin. Med.*, 51: 926-934, 1958.
 149. SCHIFF, L., BILLING, B. H. and OIKAWA, Y. Familial nonhemolytic jaundice with conjugated bilirubin in the serum. *New England J. Med.*, 260: 1315-1318, 1959.
 150. SCHLENK, F. and FISHER, A. Studies on glutamic-aspartic acid transaminase. *Arch. Biochem.*, 12: 69-78, 1947.
 151. SCHLENK, F. and SNELL, E. E. Vitamin B₆ and transamination. *J. Biol. Chem.*, 157: 425-426, 1945.
 152. SCHNEIDER, A. J. and WILLIS, M. J. Sources of variation in a standardized and a semi-micro procedure for the spectrophotometric assay of serum glutamic-oxaloacetic transaminase concentration. *Clin. Chem.*, 4: 392-408, 1958.
 153. SEVELA, M. Glutamic-oxaloacetic transaminase in the cerebrospinal fluid of man. *Experientia*, 13: 295-296, 1957.
 154. SCHABETAI, R., IGLAUER, A. and ANDERSON, D. Serum transaminase determinations as a diagnostic aid in myocardial infarction. *Brit. M. J.*, 1: 555-557, 1957.
 155. SHAY, H. and SIPLET, H. Study of chlorpromazine jaundice, its mechanism and prevention; special reference to serum alkaline phosphatase and glutamic-oxaloacetic transaminase. *Gastroenterology*, 32: 571-591, 1957.
 156. SIEKIERT, R. E. and FLEISHER, G. A. Serum glutamic-oxaloacetic transaminase in certain neurologic and neuromuscular diseases. *Proc. Staff Meet., Mayo Clin.*, 31: 459-464, 1956.
 157. SMITH, S. E., CANNACK, K. V. and DODDS, M. E. Serum transaminase determination in the differential diagnosis of jaundice. Surgical aspects. *J. Michigan M. Soc.*, 57: 1727-1729, 1958.

158. SNYDER, D. D., BARNARD, C. N., VARCO, R. L. and LILLEHEI, C. W. Serum transaminase patterns following intracardiac surgery. *Surgery*, 44: 1083-1091, 1958.
159. SOBEL, C., BERKMAN, S. and SWASS, N. Simplification of the spectrophotometric determination of glutamic-oxaloacetic transaminase in blood serum. *Am. J. Clin. Path.*, 26: 1477-1478, 1956.
160. STEINBERG, D. and OSTROW, B. H. Serum transaminase as a measure of myocardial necrosis. *Proc. Soc. Exper. Biol. & Med.*, 89: 31-34, 1955.
161. STEINBERG, D., BALDWIN, D. and OSTROW, B. H. A modified method for assay of glutamic-oxaloacetic transaminase. *J. Lab. & Clin. Med.*, 48: 144-151, 1956.
162. TONHAZY, N. H., WHITE, N. G. and UMBREIT, W. W. A rapid method for the estimation of the glutamic-aspartic transaminase in tissues and its application to radiation sickness. *Arch. Biochem.*, 28: 36-42, 1950.
163. TSITOURIS, G. and STEFANINI, M. Platelets. XXI. Glutamic pyruvic transaminase activity of human platelets. *Proc. Soc. Exper. Biol. & Med.*, 99: 40-42, 1958.
164. UMBREIT, W. W., KINGSLEY, G. R., SCHAFFERT, R. R. and SIPLET, H. A colorimetric method for transaminase in serum or plasma. *J. Lab. & Clin. Med.*, 40: 454-459, 1957.
165. WAKIM, K. G., FLEISHER, G. A., HANSON, M. L. and ARNS, R. The effect of experimental cerebral infarction on transaminase activity in serum, cerebrospinal fluid and infarcted tissue. *Proc. Staff Meet., Mayo Clin.*, 31: 391-399, 1956.
166. WALDMAN, R. K. and BORMAN, E. K. A note on serum transaminase activity after lead absorption. *Arch. Indust. Health*, 19: 431-433, 1959.
167. WANG, C. C. and APPLEHANZ, I. A preliminary report on some extraneous factors that may influence serum glutamic-oxaloacetic transaminase level. *Clin. Chem.*, 2: 249-250, 1956.
168. WEISS, T. E. and MOORE, C. B. Preliminary observations on the serum glutamic-oxaloacetic transaminase levels in gout and polycythemia vera. *Ochsner Clin. Rep.*, 3: 111-114, 1957.
169. WOOD, J. E., JR., ATUK, N. O., CAMP, J. L., III, BECKWITH, J. R. and LAURANCE, P. B. Serum glutamic-oxaloacetic transaminase and lactic dehydrogenase levels and their clinical significance. *Northwest Med.*, 57: 1447-1451, 1958.
170. WRÓBLEWSKI, F. Significance of serum enzyme alterations associated with myocardial infarction. *Am. Heart J.*, 54: 219-224, 1957.
171. WRÓBLEWSKI, F. The clinical significance of alterations in serum transaminase in hepatitis. In: *Hepatitis Frontiers*, pp. 447-465. Edited by Hartman, F. W. and Logrip, G. A. Boston, 1957. Little, Brown & Co.
172. WRÓBLEWSKI, F. The clinical significance of alterations in transaminase activities of serum and other body fluids. *Advances Clin. Chem.*, 1: 313-353, 1958.
173. WRÓBLEWSKI, F. The significance of alterations in serum enzymes in the differential diagnosis of jaundice. *Arch. Int. Med.*, 100: 635-641, 1957.
174. WRÓBLEWSKI, F. The significance of serum glutamic-oxaloacetic transaminase in experimental and clinical states. *Tr. New York Acad. Sc.*, 18: 444-450, 1956.
175. WRÓBLEWSKI, F. Unpublished observations.
176. WRÓBLEWSKI, F. and CABAUD, P. Colorimetric measurement of serum glutamic-pyruvic transaminase. *Am. J. Clin. Path.*, 27: 235-239, 1957.
177. WRÓBLEWSKI, F. and LADUE, J. S. Serum glutamic-oxaloacetic aminophorase (transaminase) in hepatitis. *J. A. M. A.*, 160: 1130-1134, 1956.
178. WRÓBLEWSKI, F. and LADUE, J. S. Serum glutamic-oxaloacetic transaminase activity as an index of liver-cell injury from cancer. A preliminary report. *Cancer*, 8: 1155-1163, 1955.
179. WRÓBLEWSKI, F. and LADUE, J. S. Serum glutamic-oxaloacetic transaminase activity as an index of liver-cell injury. A preliminary report. *Ann. Int. Med.*, 43: 345-360, 1955.
180. WRÓBLEWSKI, F. and LADUE, J. S. Serum glutamic-pyruvic transaminase in hepatic disease. *Proc. Soc. Exper. Biol. & Med.*, 91: 569-571, 1956.
181. WRÓBLEWSKI, F. and LADUE, J. S. Serum glutamic-pyruvic transaminase (SGP-T) in hepatic disease. A preliminary report. *Ann. Int. Med.*, 45: 801-811, 1956.
182. WRÓBLEWSKI, F. and MANSO, C. Effects of certain anticoagulants on serum enzyme activity. *J. A. M. A.*, 167: 2163-2168, 1958.
183. WRÓBLEWSKI, F., JERVIS, G. and LADUE, J. S. The diagnostic, prognostic, epidemiologic significance of alterations of serum glutamic-oxaloacetic transaminase in hepatitis. *Ann. Int. Med.*, 45: 782-800, 1956.
184. ZARRIELLO, J. J. Serum glutamic-oxaloacetic transaminase activity in human beings subjected to positive radial acceleratory force. *J. Aviation Med.*, 30: 97-99, 1959.
185. ZELMAN, S. and WANG, C. C. Transaminases in serum and liver correlated with liver cell necrosis in needle aspiration biopsies. *Am. J. M. Sc.*, 237: 323-334, 1959.
186. ZINKE, M. R. and LEEVY, C. M. Correlation of serum transaminases with histopathology in Laennec's cirrhosis. *Gastroenterology*, 34: 1046-1047, 1958.

The Plasma Amylase*

Source, Regulation and Diagnostic Significance

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THE blood has been known to contain amylase for more than one hundred years, and the measurement of its amylolytic activity has been used clinically for almost fifty years. Indeed, determination of the plasma amylase for the diagnosis of pancreatic disease probably represents one of the most widely studied and accepted enzyme procedures in clinical medicine. Despite these truisms, there are wide gaps in our knowledge of the amylase(s) of the blood. As recently as 1941 Somogyi [1] could state: "The origin of the diastase contained in normal blood is at present unknown." In the present paper we intend to review some of the recent evidence bearing on (1) the possible tissue sites of origin; (2) physiologic factors regulating the level of amylase in the blood; and (3) the limits of clinical usefulness of blood amylase determinations.

Definition and Action. The amylases are enzymes which hydrolyze starch (*amylum*). In addition they attack glycogen and certain degradation products (dextrins) originating from these polysaccharides. Payen and Persoz in 1833 applied the name diastase to the starch-splitting enzymes of barley malt and this term has persisted until recent time; Somogyi himself prefers it, but amylase is the widely used designation at present.

The amylases of mammalian tissues, which are of the alpha variety, have several actions: (1) the amylose and amylopectin molecules of starch are broken down by fission of α -1, 4 glucosidic linkages into α -dextrins of small molecular weight (dextrinization), with consequent liquefaction and decrease of viscosity; (2) with the rapid fall in viscosity (liquefaction), amylase alters the iodine color reactions of starch; and (3) liberates fermentable sugars, mainly maltose but some glucose as well (saccharification).

Methods of Measurement. A number of meth-

ods have been used to measure the amylase of the blood quantitatively. These have been discussed critically by Somogyi [1]. In general they depend on the properties of the enzyme just listed: (1) liquefying activity is measured by determining the decrease in the viscosity of reaction mixtures [2]; (2) dextrinogenic activity is determined by the method of Wohlgemuth [3] or some one of its modifications in which starch is depolymerized until it no longer gives the characteristic blue color with iodine; (3) methods in which residual substrate is measured, as in the method of Lagerlöf [4] in which remaining glycogen is determined nephelometrically; and (4) the saccharogenic method of Somogyi [5] based on the measurement of reducing sugars produced by hydrolysis of starch under standard conditions. This last method is the most widely used at present and is considered the most accurate. In it the amount of starch cleavage products are reported in terms of the copper-reducing power of dextrose. Thus an amylase activity of 100 units means that under the standard conditions of the determination 100 ml. of serum produces starch cleavage products which have the same copper-reducing power as 100 mg. of glucose.

Digestive Enzymes in the Blood. The presence of amylase in the blood normally is but one example of the "endocrine" secretion of the exocrine digestive glands under physiologic conditions. Gastric pepsinogen and pancreatic lipase are other examples. The question of pancreatic trypsin remains unsolved because of the problem of measurement of tryptic activity in the serum.

The meaning of this secretion into the blood of amylase is quite obscure, in part because of the probable multiplicity of sites of origin (as will be indicated in greater detail subsequently). The zymogen granules of the pancreas, as seen with

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both the light and electron microscope [6], are highly oriented towards the collecting ductular apparatus. The factors which influence the movement of even a small portion of these granules into the interstitial fluid and blood are poorly understood. An increase in resistance to outflow in the duct system of the pancreas will be reflected in the level of the circulating enzyme [7], but whether this is due solely to changes in pressure, or to changes in the permeability of the acinar cell, or to disruption of a portion of its limiting membrane is not known. The problem of what one of us has called the "exocrine-endocrine" partition of digestive enzymes [8] has been studied in greater detail in the case of gastric pepsinogen, with somewhat inconclusive results. Studying the movement of pepsinogen out of the stomach by simultaneous measurement of pepsinogen in the lumen of the stomach and its clearance by the kidney, Janowitz and Hollander concluded that about 1 per cent of pepsinogen found its way into the blood under basal conditions. They interpreted this ratio to be an expression of a physiological partition of digestive enzymes. This interpretation has not been universally accepted. Hirshowitz [9] has argued that the pepsinogen in the plasma represents enzyme derived from disintegrating cells during the normally rapid turnover of the peptic cells. The response of the urinary pepsinogen clearance to anticholinergic drugs makes it unlikely that this turnover can be the sole regulating factor in controlling plasma levels. Be this as it may, we know little at present regarding the factors governing the normal exocrine-endocrine partition of enzymes from digestive glands and other sources.

Plasma Levels and Renal Clearance of Amylase. It is reasonable to assume that the amount of amylase circulating in the serum is the net resultant of the amount entering the extracellular fluid from tissue sources and the amount being cleared by the kidney. Hepatic bile is believed to be completely free of amylolytic activity [10] and there is evidence that amylase is not reabsorbed from the intact intestine after being secreted by the salivary and pancreatic glands. Curiously, human milk contains high concentrations of amylase [11].

Since many observers have noted relative constancy of the plasma level in any one person, the twenty-four-hour urinary excretion of amylase thus represents the daily increment to the extracellular tissues and fluid, neglecting

some unknown amount metabolized. Saxon, Hinkley, Vogel and Zieve [12], in a study of some twenty-seven normal persons, noted a range of twenty-four-hour excretion levels from 792 to 4,264 units, with a mean of 1,947. The mean variation from day to day was 338 units, or approximately 20 per cent. This amount entering the plasma is extremely small in comparison, for example, with the amount of salivary and pancreatic amylase secreted into the digestive tract.

Despite the long interest in urinary amylase it is only recently that some evidence has been gathered on the manner in which the kidney clears the plasma of amylase. McGeachin and Hargan [13,14] in two studies noted that in normal subjects with moderate urine flow the amylase clearance was in the range of 1 to 3 ml./minute. When water diuresis was induced by water drinking the amylase clearance increased only slightly, and the amylase excretion remained constant over a wide range of urine flow. From their evidence they drew the conclusion that amylase appeared to be filtered but not reabsorbed, and is not a threshold substance. Further studies, however, are needed on these points.

TISSUE SOURCES OF AMYLASE

The synthesis of large amounts of alpha amylase by the pancreas of all mammalian species studied and by the salivary glands of some is widely accepted. Many older and recent studies have shown that when these sources are removed from several varieties of animals, however, amylase does not disappear from the serum or urine. Indeed when the animals recover from the operative trauma there is remarkably little change in the blood concentration [15]. The careful studies of McGeachin, Gleason and Adams [16] have shown that, except for the lower duodenal content, the tissue distribution of amylase of the pancreatectomized-salivariectomized rat is essentially the same as in the normal rat.

Although there has been some controversy in the past regarding liver amylase, the studies of these last authors [16] based on tissue/serum amylase ratios corrected for extracellular fluid fractions indicate that in the livers of rats, mice, guinea pigs and dogs there is an intracellular amylase. Striated muscle in the mouse and guinea pig also contains intracellular amylase. Roe, Smith and Treadwell [15] also have

presented evidence that the content of amylase of the liver is manyfold higher than can be accounted for by the blood in the organ, and others have clearly characterized the liver amylase [17,18].

Recently another extrapancreatic, extra-salivary amylase has been investigated. Green [19] demonstrated that cysts associated with human fallopian tubes or "tube-like" epithelium of Müllerian and mesonephric origin have an alpha amylase content 10 to 700 times greater than the normal serum. Green hypothesized that the substrate for this enzyme was the glycogen produced by the secretory endometrium. McGeachin and his associates [20] have shown that the amylase levels in the fallopian tubes of the human being, cow, rabbit and sheep are greater than the corresponding serum levels, and thus may be produced in the tubes of these species. In several other species (rat, dog, pig, guinea pig, cat and monkey) the amylase level was lower than the corresponding serum.

To increase further the list of possible tissue sources of blood amylase, Mirski in 1942 [21] (in a still to be confirmed study) reported interesting and highly suggestive evidence of an amylase in adipose tissue of the rat and rabbit, in addition to a phosphorylase. Only a small portion of the total amount of glycogen broken down by this diastase could be accounted for as glucose. The amylase converted glycogen into polysaccharides of low molecular weight (trisaccharides), which were not fermentable. On the other hand, using Van Loon's amyloclastic method for amylase, McGeachin, Gleason and Adams [16] found only small amounts of amylase in the perirenal fat of the rat.

A further incompletely studied source of amylase in animal tissues and one which may have pathologic significance are the amylase-producing bacteria in the intestine. Baker et al. have demonstrated their existence [22], and Burnett and Ness cultured starch-splitting (amylase-producing) bacteria from an inflamed human appendix [23].

These studies indicate that in addition to the pancreas and salivary glands, the liver, striated muscles, fallopian tubes and adipose tissues possibly contribute increments to the plasma content of amylase. They do not, however, shed any light as to the relative quantitative importance of each source under normal conditions.

The previously cited studies of the minimal effect of pancreatectomy on plasma levels in

many animal species (collected by Somogyi [1]), our own studies in subjects with hepatocellular disease and a pancreatectomized human subject [24], the studies of Gray, Probst and Heifetz [25] of low values in patients with liver disease all suggest that this organ probably may be the important source of the normal amounts of amylase appearing in the blood. McGeachin and Lewis [26] have recently contributed evidence which may be consonant with this view. Their studies of the electrophoretic mobility of serum amylase indicated that the amylase of normal serums is associated chiefly with the electrophoretic albumin fraction of the serum. Salivary and pancreatic amylase was associated with the gamma globulin fraction. From an analysis of eighteen serums, it appeared that less than a fourth of the amylolytic activity of the normal serum was of pancreatic or salivary origin. An interesting additional observation was noted in these studies. In every case the sum of the amylase activities of the electrophoretically separated fractions of normal serum was greater than the amylase activity of the whole serum. This suggested the presence normally of an inhibitor in the plasma. In contrast to the electrophoretic results, fractionation of serum proteins with ammonium sulfate showed that the major portion of the amylase activity was found in the globulin fraction.

PHYSIOLOGIC REGULATION OF PLASMA AMYLASE

Just as in the case of the tissue sites of origin of the amylase usually present in the blood, there has been until recently considerable uncertainty regarding the physiologic variables which regulate its level.

The enzyme, absent in newborns, usually first appears at two months, can be estimated quantitatively at three months, and according to Somogyi [1] reaches a relatively normal level in about one year. It is of interest in this context that pancreatic secretion of amylase is not well established in the first year of life.

While there are wide variations in the level of amylase in the blood of normal persons, 80 per cent have values between 80 and 150 units by Somogyi's method. For any one person the level of amylase tends to remain relatively constant.

Carbohydrate Metabolism and Normal Plasma Amylase Regulation. A correlation between blood amylase and blood sugar levels in normal and diabetic subjects has been sought in the past,

with conflicting results. Reid and Myers [27] found the blood amylase in untreated diabetic patients to be higher than in normal subjects, but within normal limits in diabetic patients treated with insulin. Somogyi, from a statistical analysis, concluded that patients with diabetes had a lower blood amylase, but ascribed this to alteration in liver function [28].

Recently we have restudied the problem in normal and diabetic subjects without evidence of abnormal exocrine pancreatic function [29]. When glucose, fructose, insulin alone or with glucose, glucagon or tolbutamid were given to these subjects, there was a prompt and significant drop (minimal 40 per cent) in the plasma level of amylase, which disappeared in two hours. Epinephrine, on the other hand, induced a marked rise in plasma levels. These changes bore no relationship to the level of blood glucose or the direction of its rise or fall. In a parallel study, Dreiling and Bierman [30] observed a good correlation between the non-esterified fatty acids of the plasma and its amylase concentration, independent of the direction of alteration of the plasma glucose content. These studies suggested that the plasma amylase falls during states of increased carbohydrate utilization, and rises following the administration of hormones that diminish utilization of carbohydrate, or in physiologic states of diminished carbohydrate utilization, as when insulin is withheld in patients with diabetes.

More recently we have observed the usual response to glucagon (lowering of plasma amylase) in a patient following recovery from total pancreatectomy. More striking was the observation that this depression of plasma amylase did not occur in subjects with hepatocellular damage [24]. This evidence is further support for the concept that the liver may be the major organ regulating the normal plasma amylase.

ACTH and Adrenal Cortical Steroids. There is evidence that the anterior pituitary may influence the level of circulating amylase, at least in the dog. Cope and his co-workers suggested that the anterior pituitary, through its influence on carbohydrate metabolism, was a major factor in the control of the blood amylase level [37]. Some recent studies by us indicate that the level of circulating plasma amylase is modified by the adrenocorticotrophic hormone and some adrenal cortical steroids [24]. The intravenous administration of 40 units of ACTH or 100 mg. of cortisol results in a biphasic reaction: a depres-

sion in level for approximately two hours followed by a marked "overshoot," associated with a modest rise in plasma glucose. A similar but less marked biphasic response occurred in subjects with hepatocellular disease. The biphasic response is difficult to explain and perhaps may be interpreted in terms of the suggested relationship between carbohydrate utilization and amylase. The initial drop may be associated with the phase of gluconeogenesis, and the secondary rise to the associated depression in glucose utilization perhaps related to postulated insulin antagonism of these compounds. Continued administration of ACTH or cortisol results in predominance of this secondary effect, the rise in blood amylase, an observation already recorded by Pfeffer and Hinton [32]. The apparent decrease in the amylase response to administration of ACTH and cortisone in patients with hepatocellular disease may be the result of a lessened ability of affected livers to derive glucose from non-carbohydrate source. The data of Dreiling, Debons, Rosenthal and Schwartz [33] on non-esterified fatty acid of the plasma (NEFA) under similar circumstances is in keeping with this interpretation.

It may be added parenthetically that these experiments on the relationship between factors affecting carbohydrate metabolism and blood amylase tend to restore a possible role for the liver amylase, which has been very much de-emphasized in recent years. However the enzyme present there presumably may participate to a limited extent in glycogenolysis, which is mostly carried out by the phosphorylases. Thus it may be that the liver's contribution to the amylase of the plasma varies with the state of carbohydrate metabolism. Stated rather crudely, the more amylase being used in the liver, the less would tend to enter the blood.

To summarize this section, available and admittedly limited evidence suggests that the salivary glands, pancreas, liver, striated muscle, adipose tissue and fallopian tubes could conceivably contribute in varying and unknown amounts to the normal level of the circulating amylase. The contribution of the pancreas is probably much smaller than has been generally supposed. In the normal subject serum amylase fluctuates rapidly in response to a variety of substances (glucose, fructose, tolbutamide, insulin, epinephrine, ACTH and cortisone) apparently because of their effect on carbohydrate metabolism in the liver. The bulk of circulating

amylase normally may thus be derived from the liver.

PATHOLOGIC ALTERATIONS OF PLASMA AMYLASE

Pancreatic Disease. Mechanism of entry of pancreatic amylase into the plasma: The problem of the exocrine-endocrine partition of digestive enzymes has already been touched upon in this review. Whatever its quantitative importance, some pancreatic amylase finds its way into the blood. Normally the concentration of amylase in pancreatic vein, peripheral veins, and thoracic lymph is roughly the same, but since the rate of lymph flow is much slower than that of venous blood the contribution of lymphatic drainage of the gland to the circulating blood must normally be rather small. However, an increase in hydrostatic pressure in the pancreatic outflow tract leads to a fairly prompt rise in the amylase concentration of the blood. Grossman has recently reviewed this aspect [34], pointing out that neither an increase in volume flow of pancreatic juice nor stimulation of pancreatic enzyme production will of themselves cause an increase in serum enzyme concentration. Rather, elevation of intraductal pressure is the important determinant. Stimulation of flow in the face of obstruction can, however, augment the entry of enzyme into the blood. Grossman is inclined to attribute this to an accentuation of the normal process of "endocrine" secretion since it occurs in the absence of histologic damage (at least by light microscopy). Experimental complete occlusion of the main pancreatic duct by clamping in the fasting anesthetized dog for one hour or less led to no rise in plasma amylase in a recent study [35], while significant rises lasting forty-eight hours followed occlusion for two or more hours. Whether this rise was due entirely to transient increased intraductal pressure is not clear; the persistence of the effect is somewhat in favor of acinar disruption. In addition to the factor of pressure rises in the outflow tract, the other source of the elevated serum amylase in clinical and experimental pancreatitis is, of course, the enzyme derived from the disrupted acinar cell. Yet attempts to compare various experimentally produced injuries show little correlation between the degree of elevation of the serum amylase and the severity of the pancreatitis. Ductular disruption or injury seemed more clearly correlated [36].

Considerable effort has been directed to a

study of the pathways of the enzyme changes which follow experimental and clinical pancreatic damage. Popper and Necheles [37], studying bile-induced pancreatitis in dogs, measured the amylase concentration of pancreatic venous blood, peripheral venous blood and thoracic lymph. After experimental injury the levels of the enzyme in the three fluids rose. After occlusion of the portal vein, no rises were noted in the lymph or the peripheral venous blood levels; with release of the occlusion, peripheral venous levels again began to rise. They interpreted this to mean that the portal vein was the main route of enzyme increases following experimental pancreatitis, although lymph drainage of the gland or peritoneum might contribute to a lesser degree.

Howard, Smith and Peters [38] in a similar although more limited study were impressed by the greater rise in blood amylase in the pancreatic vein than in peripheral veins following experimental damage in the dog. Thoracic duct occlusion did not alter this phenomenon. They too were inclined to believe that the pancreatic venous blood was the main pathway for the rise in enzyme following damage to the pancreas.

Egdahl [39], in what appears to be a definitive study, has recently reinvestigated this problem. Using the dog with bile-induced pancreatitis, he measured the venous blood flow from the uncinate lobe; simultaneous thoracic lymph and peripheral venous blood levels also were measured. The initial rise (one to three hours) after injury was due to absorption of enzymes into pancreatic venous blood. The later maintenance of rise in enzymes (both lipase and amylase) was often primarily due to lymphatic absorption of peritoneal fluid of extremely high enzyme content. This fluid resulted from the passage of highly concentrated enzyme-containing fluid through the pancreatic capsule from the pancreatic subcapsular space. It is this latter feature which is the basis of peritoneal tap as a diagnostic method in pancreatitis.

Clinical significance of plasma amylase level: In the light of the foregoing discussion two pathologic features within the pancreas therefore influence the height to which the amylase rises in pancreatic disease: (1) continued secretion against obstruction, and (2) disruption of acinar cells and ductular apparatus.

While the intraglandular pathologic processes of edema, hemorrhage and collection of inflam-

matory cells will increase intraductular obstruction, process one (just mentioned) plays a self-limited role since rapid glandular damage leads to fairly prompt impairment and even suppression of secretion, at least in experimental pancreatitis [34]. This is in contrast to gradually increasing obstruction. The amount of ductular rupture and acinar disruption is probably more important in sustaining the elevation.

It is therefore not surprising that carcinoma of the pancreas, even when obstructing the head of the gland, only rarely leads to even modest rises in plasma levels. When rises do occur they are to be ascribed rather to concomitant inflammatory disruption which frequently occurs near the periphery of a carcinoma of the pancreas.

On the other hand when disruption does occur the rise is dampened by the distribution of amylase in extracellular fluids. If it is possible to extrapolate from the rat to man, admittedly hazardous, liberation into the tissue fluids of all the amylase in 100 gm. of pancreas would lead to a rise of 4,000 units in a man weighing 70 kg. with a normal blood volume. Since the renal clearance of amylase at these abnormally high levels is not known, one cannot hazard even a guess as to how long such a rise would persist. Yet all clinicians have noted that the rise in acute pancreatitis is transient, often gone within three to four days, although persistent elevations have been reported up to ten to twenty-two days. Indeed Bockus [40] has stressed the fact that persistence of the elevation when the patient is on a good therapeutic regimen indicates a complication such as abscess or pseudocyst, with continuing inflammatory changes.

Because of the variables of ductular rupture, pancreatic disruption, secretion against obstruction, and renal clearance it is apparent that no straightforward correlation may exist between any one sample of serum for amylase and the severity of the acute pancreatitis. Nevertheless in Bockus' series of patients all those with values over 900 units did have elevation of temperature and marked leukocytosis.

In view of the transitory nature of the serum rise it is not surprising that in some patients the elevation may be missed. In one series of ninety-four attacks in seventy-eight patients, 11 per cent had normal values persistently, although two of these nine died of the disease. In 42 per cent in this series the elevation was less than five times the normal (500 mg./100 ml.); while in 47 per

cent the values were over 500 [40]. In Richman's series the values ranged between 190 to 575 units [41].

In this context the study of the electrophoretic behavior of serum amylase by McGeachin and Lewis [26] already cited is of interest. In a study of the serums of nine patients with pancreatitis (no clinical details given) the rise in plasma amylolytic activity was predominantly due to the rise in the fraction associated with γ globulin, although there was a slight increase in the albumin fraction as well. In these serums the sum of the amylolytic activity of the fractions equalled the activity of the serum, and the postulated inhibitor was not demonstrable. These authors make the interesting suggestion that the rise in pancreatitis may be due not only to an increase of pancreatic amylase but also to the removal of an inhibitor which is non-dialyzable and probably a protein.

The diagnostic use of serum amylase in diagnosing acute pancreatitis may be limited by two other factors which must be taken into consideration. These are the prior administration of opiates and the effects of renal insufficiency.

Effect of Opiates on Serum Amylase Levels. Mention has already been made of the effect of increases in intraductal pressure in facilitating the entry of pancreatic amylase into the plasma. Morphine and related compounds are known to increase pancreatic ductular pressure by a direct effect on duodenal musculature and the sphincter of Oddi. Burke and his associates [42] reported a rise in serum amylase in three normal subjects following 16 mg. administration of morphine sulfate. These rises, however, were well within the normal level. Studying the effect of codeine, in rather large doses, 2 gr. (0.13 gm.), Gross and his co-workers [43] observed a significant rise in serum amylase level in five of twenty-seven subjects, the highest rise being up to 4,000 units. The plasma level was still elevated in two patients (800, 533) twenty-four hours after the administration of codeine. Wapshaw [44] compared the effect of 16 mg. of morphine administered during the fasting state, one hour after a meal, and following administration of a parasympathomimetic drug and food. Eighty-four subjects were studied; 18 per cent had a significant rise in the fasting state, the highest value being 530. Twenty-two per cent had a rise after taking morphine and eating a meal, the highest value being 914 units; while 69 per cent of those studied had a rise after ingestion of food,

morphine and a parasympathomimetic drug, the highest value reaching 1600 units. Although Pfeffer and his associates [45] were not impressed by the effect of morphine *per se*, Bogoch, Roth and Bockus [46] noted rises in eight of forty-one patients given 16 mg. of morphine in the fasting state; three of these rises were in the range of five times the normal level considered by these authors to be diagnostic of pancreatitis. In general these rises occurred within five hours and some elevations persisted for the first twenty-four hours. The clinical implication of these limited studies is quite obvious, although occasionally overlooked.

Renal Insufficiency. It has been known for a long time that the level of blood amylase may rise due to diminished renal excretion, although, as already indicated, the exact mechanisms of renal clearance of the enzyme is not clearly established. Impaired renal function may thus be associated with retention of the enzyme in the plasma and diminished excretion in the urine. Indeed at one time the ratio of blood to urinary amylase was proposed as a measure of renal function. Myers and Killian in 1917 [47] pointed out that in a rough way plasma amylase rose parallel with the urea concentration in chronic renal insufficiency, although not to the height encountered in acute pancreatitis. In a clinical study Heifetz and his colleagues [48], reporting on 111 cases of renal insufficiency, noted seventy-nine patients with levels above 200 Somogyi units. The majority of cases were in the range of 200 to 500, with 13.5 per cent of values above 500 units, two above 1,000. More recently Meroney and his associates [49] observed the behavior of the blood amylase in some six patients with acute renal insufficiency of varied etiology. Using Van Loon's method (normal 60 to 160 units) the values were clearly elevated in all six patients, the highest value in each patient ranging from 380 to 1,388, but with no straightforward relationship to the blood urea nitrogen. When the chemical and clinical aspects of uremia were favorably modified by hemodialysis the level of amylase was not consistently affected. Sachar and Weinhaus [50] have cautioned that the plasma amylase may be consistently elevated without obvious evidence of renal impairment, and yet be due to diminished renal excretion. They have stressed the ratio between the hourly urinary clearance and the plasma level which is above unity in normal subjects. More recently, however, Gross and

his colleagues [51], in a preliminary report of what appears to be a careful study of sixty-three azotemic subjects, have de-emphasized the importance of renal insufficiency in elevating the serum amylase. But it should be noted that the use of Somogyi's method gave an upper limit of normal of 320 units in their hands. They observed only borderline elevations of amylase in two patients, and normal values in forty of forty-two azotemic patients with chronic renal disease. In twenty subjects with extrarenal azotemia there was but one borderline elevation, and 19 normal levels. In one subject with severe acute renal insufficiency treated effectively with hemodialysis (the blood urea nitrogen fell from 444 to 198 mg. per cent), the serum amylase values remained normal throughout.

Blood Amylase Responses to Drugs in Pancreatic Disease. Because of the limitations already discussed in the use of the fasting or unstimulated blood amylase determination in the diagnosis of acute and especially chronic pancreatitis and pancreatic cancer, considerable effort has been expended to increase the value of this determination in these disorders by using a variety of drugs, the so-called "provocative blood enzyme test." Blood amylase and lipase values have been determined at varying periods of time following administration of a variety of drugs, either singly or in combination. These latter have included (1) stimulation of flow of pancreatic fluid (secretin); (2) stimulation of enzyme production or release: methacholine (Mecholyl®), bethanechol (Urecholine®), and recently pancreozymin, the enzyme-stimulating hormone; and (3) increasing pancreatic outflow resistance by administration of morphine.

Physiologically, as has been pointed out, drugs of group 1 or 2 in the normal subject do not alter plasma enzyme levels. However, naturally occurring obstruction or morphine-induced sphincter resistance can increase the plasma enzyme level derived from the pancreas, since stimulation of flow by group 1 drugs in the presence of obstruction will raise intraductal pressure, and drugs of group 2 will increase the amount of enzymes available for entry into the plasma in the presence of increased intraductal pressure.

The use of these tests in patients with pancreatic disorders is limited by at least three variables: (1) resistance to outflow either at the sphincter of Oddi or obstruction within the pancreas; (2) the degree of inflammatory changes

with the acinar tissue, which may influence the naturally occurring exocrine-endocrine partition; and (3) the amount of functionally active glandular tissue (secretory mass).

Clinically these tests are limited by the need to find clear-cut differences between normal and abnormal glands: (1) The stimulant should invariably induce a significant rise in normal subjects. Failure to induce such a rise would then point to pancreatic insufficiency, either inflammatory or neoplastic. (2) Or, the stimuli should cause no rise in normal subjects, with a clear-cut rise due to intraglandular obstruction, both of neoplastic or inflammatory origin.

The literature on these provocative tests up to the introduction of pancreozymin has been summarized by Dreiling and Richman [52]. While individual workers have obtained either of the two postulated responses in some patients by varied combinations of drugs, review of this extensive material discloses much variation and overlap of results. A study of 192 patients with and without pancreatic disease at the Mount Sinai Hospital, using secretin, morphine and Mecholyl alone, or combinations of secretin and morphine, Mecholyl and secretin, or secretin together with morphine and Mecholyl, revealed small transient elevations in blood amylase, but no consistent pattern was demonstrated either in normal subjects or in patients with pancreatic disease. There thus exists at present a marked divergence of opinion as to the value of these provocative tests of pancreatic disease.

With the availability of pancreozymin, the intestinal hormone which stimulates pancreatic enzyme secretion, Howatt and his colleagues [53] introduced the use of secretin and pancreozymin as a stimulus for a new provocative serum test, with somewhat more consistent elevation in disease states when the secretory mass remained intact. More recently Sun and Shay [54] were able to elicit significant serum responses in 50 per cent of patients with pancreatic disease. Our own unreported experience with pancreozymin in seventy-five patients has been no better than previous workers with other stimuli.

Limitation of the Use of Amylase Determinations in Intra-abdominal Disease. The determination of the plasma amylase remains the single most important laboratory aid in the diagnosis of acute pancreatitis or acute exacerbations of chronic pancreatitis. The important limitations in its use are not the effect of opiates or of renal impairment, but the elevations in amylase

associated with other intra-abdominal disorders which may enter into the often difficult differential diagnosis. These considerations have become increasingly vital since all observers are agreed that surgical intervention should be avoided in pancreatitis if at all possible.

Perforated Peptic Ulcer. Modest rises in plasma amylase with perforated peptic ulcer have been known for some time. Probstein, Wheeler and Gray [55] had observed that a posterior penetrating duodenal or gastric ulcer occasionally was associated with some increase in plasma amylase activity. In their experience these rises were associated with direct involvement of the pancreas by contiguity of the penetrating ulcer. This may reflect edematous compression of the pancreatic duct as well as localized pancreatitis.

Later, however, it became clear that free perforations of gastroduodenal ulcers, not at all in contact with the pancreas, also were associated with moderate elevation of the plasma amylase. Musgrove [56] reported three such patients in 1950; and Burnett and Ness [23] in a study of thirty-one perforated peptic ulcers found eight subjects whose values by the Somogyi method were over 400 mg. per cent; indeed two were over 1,000, one reaching 1,600. These extremely high values are the exception. Raffensberger [57] in 1951 collecting some twenty-one patients with elevated serum amylase without intrinsic pancreatic disease, noted five such amylase elevations in perforated peptic ulcers, the highest being 326 units. Amerson and his colleagues [58], restudying the problem, recently noted that 22 per cent of forty-one patients with acute perforations of gastric or duodenal ulcers had elevations above the normal, the three highest being 449, 486 and 488 mg. per cent.

These rises in amylase appear to be due to absorption of enzyme from the peritoneal cavity. Pemberton, Grindlay and Bollman [59] studied the serum amylase values after experimental perforation of the duodenum in dogs. These perforations were created in the duodenum from the postpylorus region to 1 cm. below the entrance of the pancreatic duct. Four of fourteen animals (29 per cent) had moderate elevations of from 24 to 120 per cent of control levels following such experimental perforations.

Intraperitoneal injection of pancreatic juice in five normal dogs was followed by a prompt rise in plasma levels. This occurred also in five dogs with pancreatic atrophy secondary to pancreatic duct ligation and in two pan-

createctomized dogs, the presumption being that lymphatic absorption of enzyme from the peritoneum was responsible for the rise. This presumption is strengthened by the finding of Amerson and his colleagues that the majority of patients with perforated ulcers who were studied by peritoneal aspirations had fluids whose amylase activity was higher than that of the plasma. In twenty-six such studies, the peritoneal fluid was greater than 200 in seventeen, and over 1,500 mg. per cent in three (12 per cent).

Intestinal Obstruction. A significant rise in the amylase activity of the blood occurs in some few cases of intestinal obstruction. Burnett and Ness [23], reporting on thirty-five patients, and using Somogyi's method, found nineteen patients whose values were above their normal range of 62 to 177 (mean $115 \pm$ standard deviation 33). Of these two were very high: one to 1,600, and one to 2,000. Both of these patients had extensive devitalization of small bowel accompanying the obstruction. These authors postulated that the amylase of the intestinal content was absorbed either by capillaries or by lymphatics of the damaged bowel or from the peritoneum after seeping through the intestinal wall. In addition to the pancreatic increment to the intestinal content of amylase, the intestinal cells of the crypts of Lieberkühn might have contributed some desquamated amylase, and amylase-producing bacteria have been cultured from the gut. The importance of these organisms in this connection has not been established.

In experimental studies on the dog (in which normal amylase values are about ten times higher than in man) Boyd and Byrne [60] noted a rise in serum amylase in the majority of their animals following complete mechanical intestinal obstruction provided that the pancreatic juice entered the intestine above the site of obstruction. This rise was not correlated with either high or low intestinal obstruction, feeding or starvation, dehydration or fluid replacement. The elevation could not be ascribed to an increase in the amylase content of the abdominal fluid which intestinal obstruction may occasionally produce, since the amylase content of this fluid when present was never greater than that of the serum if intestinal continuity was intact.

Hiatt [61] noted that, in general, simple mechanical obstruction in the dog, without strangulation, did not elevate the serum amylase levels. However, strangulating obstruction with

necrosis of mucosa was followed by significant rises in plasma amylase values; rises which occurred in two depancreatectomized dogs as well. Since Hiatt found little evidence of amolytic activity of intestinal bacteria, he ascribed the rise to absorption of enzyme seeping through the devitalized bowel wall into the peritoneum. The peritoneal fluids often had values above the plasma. Both groups of workers found no evidence of pancreatitis resulting from their maneuvers.

This situation should be clearly differentiated from the variety of pancreatitis produced by Pfeffer and his colleagues [62] by closed loop obstruction of the duodenum in the area where the pancreatic duct enters. We have recently observed the clinical counterpart of this entity in several patients.

Disorders of the Liver and Biliary Tract. Although he has been reluctant to assume that the liver is a source of plasma amylase, Somogyi as far back as 1934 pointed out that low values seemed to be indicative of impaired hepatic function. In a large series (170) of healthy persons, Somogyi [63] found that only 4 per cent had values below 60; in 120 of 1,209 "normals" drawn from hospital patients 13 per cent had values in this low range. In contrast, in 235 patients with diseases of the liver and bile ducts [25] more than 50 per cent had values below 60 units. This admittedly heterogenous group included single or multiple abscesses of the liver, acute hepatitis, malignancies of the liver and bile ducts, cirrhosis, and some instances of acute yellow atrophy and toxic hepatitis. A similar large number of abnormally low values occurred in acute cholecystitis, but not in chronic disease of the gallbladder. These findings were interpreted by Gray, Probst and Heifetz [25] as evidence of impaired liver function. Other authors have reported some similar findings. Since jaundice *per se* is known not to influence the serum amylase, these observations are consistent with the suggestion advanced earlier that the liver may be a significant source of the plasma amylase, and production of the enzyme is dependent on the functional integrity of the liver cell.

On the other hand there are frequent reports in the literature of elevated amylase values in acute obstruction of the common bile duct and in some patients with disease of the gallbladder. It is convenient to assume that these high values simply represent concomitant involvement of

the pancreas. This may indeed be the case, but the evidence on this point is frequently incomplete.

The Postoperative Patient. In a series of eighty-five patients operated upon chiefly for upper abdominal conditions, Perryman and Hoerr [64] observed twenty-seven (32 per cent) who had levels of amylase of 500 or over by a method whose upper limit of normal was 200. Dunphy, Brooks and Achroyd [65] have stressed this syndrome of postoperative pancreatitis which they believe follows even rather minor trauma to the blood supply of the pancreas in the course of biliary or gastric surgery. It may also be due to proximal duodenal loop obstruction following gastrectomy, which leads to secondary rise of intraductal pressures. These rises in amylase are probably to be ascribed to pancreatic injury, but it is difficult to rationalize the occasional rise in plasma amylase which follows lower abdominal operations apparently quite remote from the pancreas.

Ruptured Ectopic Pregnancy. Evidence has been cited that the human fallopian tube may secrete an alpha amylase. It is of interest therefore that a markedly elevated serum amylase has been reported in association with a ruptured fallopian tube pregnancy [66]. This appears to be an interesting area for further clinical observations.

Non-abdominal Trauma. The lability of the factors regulating the plasma amylase is emphasized by some recent studies on the effects of shock and of trauma to areas other than the abdomen. Howard, Frawley and Artz [67] noted that in some nine of fourteen wounded soldiers in the Korean War there was a tendency of the plasma amylase to fall below normal levels and to remain low for as long as three to five days after surgery. They were inclined to attribute this to damage to the liver occurring later. On the other hand Smolek, Nash and Ninecourt [68], studying blood amylase by Somogyi's method in 109 subjects with cerebral trauma, noted that twenty-six subjects had elevated values, some reaching as high as 950 mg. per cent. These authors speculated that this increase might have some relation to the hyperglycemic syndrome of injuries to the head. Their patients included subjects with concussions, with and without skull fractures or other fractures, and intracranial hematomas. They cite the work of Lobello [69] indicating a rise in plasma amylase in experimental shock due to burns and

trauma. The effects of ACTH and cortisone, already mentioned, may be involved in this rise.

SUMMARY

The total amylolytic activity of the blood appears to be the sum of the activities of several alpha amylases of diverse origin. The salivary glands, pancreas and liver are the likely important tissue sources of the normal blood amylase, although the fallopian tubes, striated muscle and even adipose tissue may possibly contribute in varying degrees. Until methods for specifically labeling the amylase of these tissues are developed this remains conjectural. However, under physiologic conditions the contribution of the pancreas and salivary glands is probably smaller than has been hitherto considered, that of the liver considerably greater.

The serum amylase responds quickly and transiently to a variety of substances and hormones which affect carbohydrate metabolism in the liver. As a rough generalization, states of increased carbohydrate utilization are associated with lowered plasma amylase levels. These alterations are not dependent on pancreatic function.

In pathologic states of the salivary glands and pancreatic glands, the serum amylase is increased by contributions from these organs. Secretion against obstruction, rupture of ductular apparatus and glandular tissue result in the appearance of variable amounts of amylase in the peripheral blood by way of the venous drainage of the pancreas, and by lymphatic absorption from the peritoneum.

Plasma amylase determinations remain the most important laboratory aid in the diagnosis of acute pancreatitis or of acute exacerbations of chronic pancreatitis. The peripheral levels of amylase do not accurately mirror the severity of the pathologic process. Administration of opiates may contribute to the elevation. The role of renal clearance of the enzyme is not completely elucidated.

Perforated peptic ulcer and intestinal obstruction with some necrosis of bowel wall may occasionally result in elevated serum amylase levels derived from the peritoneal absorption of enzyme.

REFERENCES

1. SOMOGYI, M. Diastatic activity of human blood. *Arch. Int. Med.*, 67: 665, 1941.

2. DAVISON, W. C. A viscosimetric method for the quantitative determination of amylase. *Bull. Johns Hopkins Hosp.*, 37: 281, 1935.
3. WOHLGEMUTH, J. Über eine neue Methode zur quantitativen Bestimmung der diastatischen Ferments. *Biochem. Ztschr.*, 9: 1, 1908.
4. LAGERLÖF, H. O. Pancreatic Function and Pancreatic Disease Studied by Means of Secretin. New York, 1942. Macmillan.
5. SOMOGYI, M. Micromethods for estimation of diastase. *J. Biol. Chem.*, 125: 399, 1938.
6. PALAY, S. L. The Morphology of Secretion. In *Frontiers in Cytology*. Edited by S. L. Palay. New Haven, 1958. Yale University Press.
7. GIBBS, G. E. and IVY, A. C. Early histological changes following obstruction of pancreatic ducts in dogs: correlation with serum amylase. *Proc. Soc. Exper. Biol. & Med.*, 77: 251, 1951.
8. JANOWITZ, H. D. and HOLLANDER, F. The exocrine-endocrine partition of enzymes in the digestive tract. *Gastroenterology*, 17: 591, 1951.
9. HIRSHOWITZ, B. Pepsinogen, its origin, secretion and excretion. *Physiol. Rev.*, 37: 475, 1957.
10. POPPER, H. L. Pankreassaft in den Gallenwegen. *Arch. f. klin. Chir.*, 175: 660, 1933.
11. KUTTNER, M. and SOMOGYI, M. Diastase in milk. *Proc. Soc. Exper. Biol. & Med.*, 32: 564, 1934.
12. SAXON, E. I., HINKLEY, W. C., VOGEL, W. C. and ZIEVE, L. Comparative value of serum and urinary amylase in the diagnosis of acute pancreatitis. *Arch. Int. Med.*, 99: 607, 1957.
13. McGEACHIN, R. L. and HARGAN, L. A. Renal clearance of amylase. *Proc. Soc. Exper. Biol. & Med.*, 89: 129, 1956.
14. McGEACHIN, R. L. and HARGAN, L. A. Amylase clearance and excretion during water diuresis. *Proc. Soc. Exper. Biol. & Med.*, 95: 341, 1957.
15. ROE, J. H., JR., SMITH, B. W. and TREADWELL, C. R. Blood, urine and tissue amylase in depancreatized rats. *Proc. Soc. Exper. Biol. & Med.*, 87: 79, 1954.
16. McGEACHIN, R. L., GLEASON, J. R. and ADAMS, M. R. Amylase distribution in extrapancreatic extrasalivary tissues. *Arch. Biochem.*, 75: 403, 1958.
17. HOLLANDER, L. The amylase system of the liver. *Science*, 79: 17, 1934.
18. HOLMBUGH, O. Studien über Leberamylase. *Ztschr. f. physiol. Chem.*, 134: 68, 1924.
19. GREEN, C. L. Identification of alpha amylase as a secretion of the human fallopian tube, and "tube-like" epithelium of müllerian and mesonephric duct origin. *Am. J. Obst. & Gynec.*, 73: 402, 1957.
20. McGEACHIN, R. L., HARGAN, L. A., POTTER, B. A. and DAUS, A. T., JR. Amylase in fallopian tubes. *Proc. Soc. Exper. Biol. & Med.*, 99: 130, 1958.
21. MIRSKI, A. Metabolism of adipose tissue in vitro. *Biochem. J.*, 36: 232, 1942.
22. BAKER, F., NASR, H., MORRICE, F. and BRUCE, J. Bacterial breakdown of structural starches and starch products in the digestive tract of ruminant and non-ruminant mammals. *J. Path. & Bact.*, 62: 617, 1950.
23. BURNETT, W. and NESS, T. D. Serum amylase and acute abdominal disease. *Brit. M. J.*, 2: 770, 1955.
24. DREILING, D. A., ROSENTHAL, W. K., KASS, M. and JANOWITZ, H. D. Relationship between blood amylase and factors affecting carbohydrate metabolism. II. The influence of ACTH, hydrocortisone, liver disease and pancreatectomy. *Am. J. Digest. Dis.*, NS 4: 731, 1959.
25. GRAY, L. H., PROBSTEN, J. G. and HEIFETZ, C. J. Clinical studies in blood diastase. 1. Low blood diastase as an index of impaired hepatic function. *Arch. Int. Med.*, 67: 805, 1941.
26. McGEACHIN, R. L. and LEWIS, J. P. Electrophoretic behavior of serum amylase. *J. Biol. Chem.*, 234: 795, 1959.
27. REID, E. and MYERS, V. C. Studies on animal diastase. IV. The effect of insulin on the diastatic activity of the blood in diabetes. *J. Biol. Chem.*, 99: 607, 1933.
28. SOMOGYI, M. Blood diastase in health and diabetes. *J. Biol. Chem.*, 134: 315, 1940.
29. DREILING, D. A., JANOWITZ, H. D., MARSHALL, D. and HAEMMERLI, P. Relationship between blood amylase and factors affecting carbohydrate metabolism. I. The regulation of blood amylase levels in subjects without pancreatic disease. *Am. J. Digest. Dis.*, NS 3: 214, 1958.
30. DREILING, D. A. and BIERMAN, E. L. Correlation between plasma amylase activity and concentration of plasma non-esterified fatty acids (NEFA). *Proc. Soc. Exper. Biol. & Med.*, 95: 496, 1957.
31. COPE, O., HAGERSTIMIER, A. and BLATT, H. The activity of the blood serum amylase in the hypophysectomized dog. *Am. J. Physiol.*, 182: 428, 1938.
32. PFEFFER, R. E. and HINTON, J. W. Some relationships between adrenal medullary and cortical substances and exocrine secretion of the pancreas. *Gastroenterology*, 31: 746, 1957.
33. DREILING, D. A., DEBONS, A. F., ROSENTHAL, W. S. and SCHWARTZ, I. L. Studies on the role of glucagon, ACTH and hydrocortisone in the regulation of the plasma level of non-esterified fatty acid (NEFA) in man. *Clin. Res.*, 7: 251, 1959.
34. GROSSMAN, M. I. Experimental pancreatitis. *Arch. Int. Med.*, 96: 298, 1955.
35. BYRD, B. F. and SAWYER, J. L. The reflection of temporary pancreatic duct occlusion in serum amylase levels of the experimental animal. *Surg., Gynec. & Obst.*, 105: 207, 1957.
36. THISTLEWAITT, J. R. and HILL, P. H. Serum amylase levels in experimental pancreatitis. *Surgery*, 31: 495, 1952.
37. POPPER, H. L. and NECHELES, H. Pathway of enzymes into the blood in acute damage of the pancreas. *Proc. Soc. Exper. Biol. & Med.*, 43: 220, 1940.
38. HOWARD, J. M., SMITH, A. K. and PETERS, J. Acute pancreatitis. Pathway of enzymes into the blood stream. *Surgery*, 26: 161, 1949.
39. EGDAHL, R. H. Mechanism of blood enzyme changes following the production of experimental pancreatitis. *Ann. Surg.*, 148: 389, 1958.
40. BOCKUS, H. L., KALSEN, M. H., ROTH, J. L. A., BOGOCH, A. L. and STEIN, G. Clinical features of acute inflammation of the pancreas. *Arch. Int. Med.*, 96: 308, 1955.
41. RICHMAN, A. Acute pancreatitis. *Am. J. Med.*, 21: 246, 1956.
42. BURKE, J. O., PLUMMER, K. and BRADFORD, S. Serum amylase response to morphine, mecholyl

- and secretin as a test of pancreatic function. *Gastroenterology*, 15: 699, 1950.
43. GROSS, J. B., COMFORT, M. W., MATHIESON, D. R. and POWER, M. H. Elevated value for serum amylase and lipase following the administration of opiates: a preliminary report. *Proc. Staff Meet. Mayo Clin.*, 26: 81, 1951.
 44. WAPSHAW, H. Pancreatic side effects of morphine. *Brit. M. J.*, 1: 373, 1953.
 45. PFEFFER, R. B., STEPHENSON, H. E. and HINTON, J. W. The effect of morphine, demerol and codeine on serum amylase values in man. *Gastroenterology*, 23: 482, 1953.
 46. BOGOCH, A., ROTH, J. L. A., BOCKUS, H. L. The effects of morphine on serum amylase and lipase. *Gastroenterology*, 26: 697, 1954.
 47. MYERS, C. V. and KILLIAN, J. A. Studies on animal diastases. I. The increased diastatic activity of the blood in diabetes and nephritis. *J. Biol. Chem.*, 29: 179, 1917.
 48. HEIFETZ, C. J., PROBSTEN, J. G. and GRAY, L. H. Clinical studies on blood diastase. II. Significance of blood diastase. *Arch. Int. Med.*, 67: 819, 1941.
 49. MERONEY, W. H., LAWSON, N. L., RUBIN, M. E. and CARBONE, J. V. Some observations of the behavior of amylase in relation to acute renal insufficiency. *New England J. Med.*, 255: 315, 1956.
 50. SACHAR, L. A. and WEINHAUS, R. Misinterpretation of elevated blood diastase due to impaired renal clearance. *Arch. Surg.*, 73: 305, 1956.
 51. GROSS, J. B., PARKIN, T. W., MAHER, F. T. and POWER, M. H. Values of serum amylase and lipase in azotemic persons with chronic renal disease and in those with extrarenal azotemia. Program of the Am. Gastroenterol. Assoc., p. 85, Atlantic City, June 1959.
 52. DREILING, D. A. and RICHMAN, A. Evaluation of provocative blood enzyme tests employed in the diagnosis of pancreatic disease. *Arch. Int. Med.*, 94: 197, 1954.
 53. HOWATT, H. T. Investigation of pancreatic dysfunction in modern trends in gastroenterology, 2nd series, edited by F. A. Jones, New York, 1958. Paul B. Hoeber.
 54. SUN, D. C. H. and SHAY, H. Pancreozymin-secretin test—the combined study of serum enzymes and duodenal contents in the diagnosis of pancreatic disease. Program of the Am. Gastroenterol. Assoc., p. 35, Atlantic City, June 1959.
 55. PROBSTEN, J. G., WHEELER, P. H. and GRAY, L. H. Perforated peptic ulcer: its differentiation from acute pancreatitis by blood diastase determination. *J. Lab. & Clin. Med.*, 24: 449, 1939.
 56. MUSGROVE, J. E. Elevated serum amylase levels associated with perforated gastroduodenal lesions. *Proc. Staff Meet. Mayo Clin.*, 25: 8, 1950.
 57. RAFFENBERGER, E. C. Elevated serum pancreatic enzyme values without primary intrinsic pancreatic disease. *Am. Int. Med.*, 35: 342, 1951.
 58. AMERSON, J. R., HOWARD, S. M. and VOWLES, K. D. The amylase concentration in serum and peritoneal fluid following acute perforation of gastroduodenal ulcer. *Ann. Surg.*, 147: 245, 1958.
 59. PEMBERTON, A. H., GRINDLAY, J. H. and BOLLMAN, J. L. Serum amylase levels after acute perforation of the duodenum: a preliminary report. *Proc. Staff Meet. Mayo Clin.*, 25: 5, 1950.
 60. BOYD, T. F. and BYRNE, J. J. Serum amylase levels in experimental intestinal obstruction. *Surgery*, 45: 747, 1959.
 61. HIATT, N. Observations of the blood amylase level in the dog with experimentally produced simple and strangulated obstructions of the small intestine. *Ann. Surg.*, 149: 77, 1959.
 62. PFEFFER, R. B., STASIOR, O. and HINTON, J. W. Clinical picture of sequential development of acute hemorrhagic pancreatitis in dogs. *S. Forum*, 8: 248, 1957.
 63. SOMOGYI, M. Blood diastase as an indicator of liver function. *Proc. Soc. Exper. Biol. & Med.*, 32: 538, 1934.
 64. PERRYMAN, R. G. and HOERR, S. O. Observations on postoperative pancreatitis and postoperative elevation of the serum amylase. *Am. J. Surg.*, 88: 417, 1954.
 65. DUNPHY, J. E., BROOKS, J. R. and ACHROYD, F. Acute postoperative pancreatitis. *New England J. Med.*, 248: 445, 1953.
 66. KELLEY, M. L., JR. Elevated amylase level associated with ruptured ectopic pregnancy. *J. A. M. A.*, 164: 406, 1957.
 67. HOWARD, J. M., FRAWLEY, J. P. and ARTZ, C. P. A study of plasma amylase activity in the combat casualty. *Ann. Surg.*, 141: 337, 1955.
 68. SMOLEK, E. A., NASH, F. P. and NINECOURT, J. Blood diastase activity in cerebral trauma. *Ann. Surg.*, 138: 863, 1953.
 69. LOBELLO, F. La diastasemia in rapporto alla glicemia nello shock traumatico sperimentale. *Rev. di pat. sper.*, 16: 387, 1936.

Some Enzymologic Aspects of the Human Erythrocyte*

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THE mature human red blood cell (RBC) is unique among the cells of the body in that its cellular integrity is preserved in spite of the absence of a nucleus and of cytoplasmic subcellular particles. The RBC has become adapted to a cellular metabolism dependent almost entirely upon glycolysis, retaining only atrophied remnants of oxidative carbohydrate metabolism, of the tricarboxylic acid cycle, and of biosynthetic activities. These peculiarities are summarized in the following characterization:

1. Energy metabolism is limited largely to glycolysis and is not accompanied by extensive synthetic or degradative activity; there is no significant oxygen consumption.
 - a. Glycolysis in RBC has several special features: (α) maintenance of a high steady-state concentration of 2,3-diphosphoglycerate (2,3-DPGA), (β) special regulatory mechanisms, (γ) inactive adenosine triphosphatase (ATPase) in the intact cell, (δ) a non-functional oxidative pathway for glucose catabolism.
 - b. Certain regulatory mechanisms in glycolysis are linked to the function of the glycolipoprotein envelope of the cell.
2. The life span of the cell is finite and the age of the cell determines vulnerability to destructive processes.
3. The human RBC normally has a discoid, biconcave shape, no nucleus, and no cytoplasmic particles.
4. RBC of primates are freely permeable to glucose in contrast to those of other mammals; glucose enters the RBC instantaneously independent of extracellular concentration (within limits) [1].

The human RBC behaves in many respects like other cells of the body, often reflecting the

general metabolic state of the organism [2]. In spite of loss of the nucleus the RBC carries genetic information like other somatic cells, in consonance with the genotypic characteristics of the person. The metabolic activity of the RBC is therefore subject to the controlling action of genes.

In the RBC glucose is converted to lactic acid by a series of enzyme-catalysed reactions similar to those generally found in the cytoplasmic fluid of somatic cells, but glucose is not oxidized to carbon dioxide except under special circumstances to be discussed later.

The mature human RBC is devoid of structured subcellular units, such as microsomes or mitochondria, but the stromal fraction enveloping the RBC represents an ordered cytoarchitectural element which may serve as a metabolic compartment [3,4] and which contains firmly bound enzymes and metabolites. Because of the stromal affinity for certain enzymes there is a differential distribution of enzymes between the interior and the structural stromal portion of the RBC, which may be of considerable metabolic importance in regulating the traffic of metabolites flowing across the RBC membrane.

Representatives of the class of hydrolytic enzymes are often found only in the stromal portion of the RBC and are often inactive in the intact RBC because of their high affinity for the lipoprotein matrix of the stroma. When the stromal structure is damaged in the preparation of a hemolysate, dissociation of enzymes from the matrix takes place and enzymatic activity becomes detectable. The following may be cited as examples of the stroma-bound type of enzymes: (a) peptidases [5-7], acetyl cholinesterase (AChE) [8-10], adenosinetriphosphatase (ATPase) [11-16], diphosphopyridine nucleotidase (DPNase) [17-19]. The nature and location of the binding sites for these enzymes at the

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stromal surface remain to be established. Although it is unknown whether there is a functional significance in the location of these enzymes at or near the cell surface, AChE has been thought to play an important role in regulating cell permeability [20–22]; proteolytic enzymes at the cell surface have been linked causally to RBC senescence [23]; and minor structural changes at the site of ATPase binding with activation of ATPase have been implicated in shifts of equilibria between intermediary metabolites of glycolysis [24]. Although such postulates have some basis in fact, their validity remains to be established rigorously.

Certain enzymes of the glycolytic pathway may be involved in the transport of metabolites into and out of the RBC when such enzymes are located at the cell surface [25]. Thus glyceraldehyde-3-phosphate dehydrogenase (GPD) could play a role in the transport of inorganic phosphate (P_i) across the membrane of the RBC since GPD catalyses the reaction by which P_i enters the glycolytic pathway. This postulate is supported by the finding that P_i enters the interior of the RBC as organically bound phosphate and, as such, is a precursor of ATP, whereas intracellular P_i arises from the breakdown of ester phosphates and not from extracellular P_i that has diffused into the cell [3,26].

It may be postulated that the stromal fraction of the RBC functions as an integral metabolic unit [3,4] which participates actively in the transport of metabolites and in the regulation of concentration gradients. Some evidence for such an hypothesis is found in the observation that the isotope concentration of 2,3-diphosphoglycerate (2,3-DPGA) in the stromal fraction differs from the isotope concentration of 2,3-DPGA isolated from the stroma-free fraction after the RBC has been incubated in the presence of P_i^{32} [3].

It has been suggested [4] that the ATPase system may participate in the transport of P_i out of the cell and that this enzyme system, in conjunction with GPD, could negotiate phosphate exchange into and out of the RBC.

Some of the stromal enzymes exhibit changes similar to those of enzymes in the interior of the RBC with respect to senescence. The AChE activity of RBC decreases progressively with increasing age [27], although the rate of change differs from that found for glucose-6-phosphate dehydrogenase (G6PD) and GPD [28,29]. Such differences in rates of change might be predicted since inactivation of structure-bound

enzymes should involve environmental and structural parameters different from those affecting enzymes soluble in the intracellular fluid. When aging of RBC is accelerated as a result of pathological conditions, e.g., in hemolytic disease, the stromal compartment may assume considerably greater than normal importance in the intermediary metabolism of RBC, particularly when the pathological process directly involves the surface of the RBC. For example, the coating of the RBC surface with isoimmune antibodies may alter the environment of the enzyme-stromal-lipoprotein complexes so as to bring about inactivation of critical enzymes at an abnormally rapid rate [120]. Similarly, an attack on critical enzyme-binding sites at the RBC surface by viruses [30] or by products of bacterial metabolism [37] may affect surface-contained enzymes and lead to early disintegration of the cell. Under such pathological conditions the rate of cell destruction ceases to be solely a function of the age of the cell, but is a function also of the degree of damage to the cell surface.

An important quantitative aspect deserves consideration in connection with pathological states affecting the integrity of the RBC, namely, how large a change in spatial parameters at the RBC surface or how great a change in enzymatic activity can be tolerated by the RBC before the integrity of the cell is threatened. The margins of safety within which a change in enzyme concentration would not affect the integrity of the cell would have to be known in order to determine the diagnostic applicability and specificity of the enzymatic changes under consideration. The available information concerning surface phenomena involving enzymatic changes is fragmentary, and evaluation of this problem therefore must be postponed. However, since there is some evidence which implicates changes in stromal organization as the primary cause of red cell destruction [24], further exploration of stromal enzyme systems and their immediate metabolic environment should be rewarding.

The enzymes of the interior of the RBC have been studied widely and are readily accessible to investigation since they are not bound to structured material.

Some Aspects of Glycolysis in the RBC. The RBC of primates are freely permeable to glucose (up to approximately 600 mg. per cent glucose in plasma) and the initial phosphorylation of glucose occurs in the interior of the cell. The

TABLE I
THE ENZYMES OF GLUCOSE METABOLISM IN THE HUMAN ERYTHROCYTE

| Enzyme | Enzymatic Activity* | Reference | Substrate | Concentration of Substrate† | Reference |
|--|-----------------------|-----------|---|-----------------------------|--------------|
| Glucokinase | 20 | [102] | Glucose | 37 | [104] |
| Glucose-6-phosphate isomerase | 9,000 | [101] | Glucose-6-phosphate | 0.73-0.91 | [46] |
| Phosphofructokinase | 384 | [44] | Fructose-6-phosphate | 0.09-0.18 | [46] |
| Aldolase | 12.7 | [103] | Fructose-1,6-diphosphate | 0.54-1.10 | [46] |
| Triose phosphate isomerase | 46,130 | [28] | Glyceraldehyde-3-phosphate | ? | |
| Glyceraldehyde-3-phosphate dehydrogenase | 2,610 | [28] | Glyceraldehyde-3-phosphate | ? | |
| 3-Phosphoglycerate-1-kinase | 1,630 | [28] | 1,3-Diphosphoglycerate | ? | |
| Monophosphoglycerate mutase | ? | | Sum of 2- and 3-phosphoglycerate | 0.45-0.73 | [46] |
| 2,3-Diphosphoglycerate mutase | ? | | 1,3-Diphosphoglycerate | ? | |
| 2,3-Diphosphoglycerate phosphatase | 2.12×10^{-2} | [109] | 2,3-Diphosphoglycerate | 32.7-46.3 | [46] |
| Enolase | 1,184 | [28] | 3-Phosphoglycerate | ? | |
| Phosphopyruvate kinase | 2,415 | [28] | Phospho-enol pyruvate | ? | |
| Lactic dehydrogenase | 10,200 | [28] | Lactic acid | 7.8 | [104] |
| Glucose-6-phosphate dehydrogenase | 848 | [28] | Glucose-6-phosphate | 0.73-0.91 | |
| 6-Phosphogluconolactone lactonase | ? | | 6-Phosphogluconolactone | ? | |
| 6-Phosphogluconate dehydrogenase | 0.66‡ | [110] | 6-Phosphogluconate | ? | |
| Phosphoribose isomerase | 27,300 to 50,000 | [81] | R-5P | Trace | [46] |
| Phosphoribomutase | 3,140 | [90] | Ribose-1-phosphate | ? | |
| Phosphoketo pentose epimerase | ? | | Ribulose-5-phosphate | ? | |
| Transketolase | 24-67 | [82] | Ribose-5-phosphate, xylulose-5-phosphate | Trace? ? | [46] |
| Transaldolase | ? | | Sedoheptulose, glyceraldehyde-3-phosphate | ? | |
| Purine nucleoside phosphorylase | 8,760 | [105] | Inosine | | |
| Adenosine triphosphatase | 5.6 | [13] | Adenosine triphosphate, adenosine diphosphate | 9.9-10.9 1.7-2.2 | [46] [46] |
| Phosphomonoesterase | 1,280 | [106] | Phenyl phosphate | | [106] |
| Diphosphopyridine nucleosidase | ? | | ? | | |
| Methemoglobin reductase | 67 | [28] | Methemoglobin | 1% of total hemoglobin | [111] |
| Glutathione reductase | 670 | [50] | Oxidized glutathione (GSSG) | 5,000 | [67] |
| Phosphoglucomutase | 281 | [107] | Glucose-1-phosphate | None detected | [46] |
| Adenylate kinase | 328,000 | [108] | Adenosine diphosphate | 1.7-2.2 | |

TABLE I (Continued)
THE ENZYMES OF GLUCOSE METABOLISM IN THE HUMAN ERYTHROCYTE

| Enzyme | Enzymatic Activity* | Reference | Substrate | Concentration of Substrate† | Reference |
|---|---------------------|-----------|-----------------------------|-----------------------------|-----------|
| Galactokinase..... | 0.9 | [96] | Galactose | ? | |
| Galactose-1-phosphate uridyl transferase | 7.4 | [96] | Galactose-1-phosphate, UDPG | ? | |
| Uridine diphosphogalactose-4-epimerase..... | 2.9 | [96] | UDPGal | ? | |
| Uridine diphosphoglucose pyrophosphorylase | 10.9 | [96] | Glucose-1-phosphate | None detected | |

NOTE: The lack of quantitative information concerning the concentration of certain enzymes and metabolites in the human RBC is indicated by a question mark (?).

* The enzyme activity stated has been calculated (where necessary) from reports in the literature and is expressed in terms of μ moles of substrate utilized/hour per 10^{11} RBC.

† The endogenous substrate concentration is expressed as μ moles/ 10^{11} RBC.

‡ The activity of PNP is expressed as stated by Marks [170] in terms of the change in optical density at 340 m μ /min./ 10^6 RBC.

following enzymes catalyse the reactions of glycolysis, beginning with the initial phosphorylation of glucose and following thereafter in the order stated: hexokinase (HK), glucose-6-phosphate isomerase (GPI), phosphofructokinase (PFK), aldolase (Al), triosephosphate isomerase (TPI), GPD, phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase (En), phosphopyruvate kinase (PPK) and lactic dehydrogenase (LD). These enzymes have all been detected in the RBC and measured quantitatively, as shown in Table I [28, 32-34].

The Rapoport-Luebering Cycle. In addition to the classical reaction sequence of glycolytic reactions, the RBC of man and of some other mammals possess a supplementary cycle at the triosephosphate level, which will be referred to as the "Rapoport-Luebering Cycle" (R. L. C.), after its discoverers [35-37]. In considering the function of the R. L. C. in RBC glycolysis, the following points should be recalled: (a) endergonic reactions utilizing ATP have atrophied in mature, human RBC and (b) the ATPase system which regulates ATP and ADP concentrations in other cells of the body is functionally inactive in the intact RBC. Since the RBC utilizes more glucose than would be required for maintenance of cellular integrity, an energy-dissipating mechanism must be provided to insure continued utilization of glucose. In the absence of a system which can dispose of excess energy-rich phosphate, ATP and 1,3-diphospho-

glyceric acid (1,3-DPGA) would accumulate and the concentration of ADP and P_i would decrease, resulting in retardation or even complete cessation of glycolytic activity.

The R. L. C. is a two-step process which constitutes a channel for "wasting" energy not used by the cell, and which may be considered an adaptive effort of the RBC. In addition to safeguarding glycolysis, the R. L. C. may also function as a self-regulating system when coupled with PGK. Such a system is sensitive to changes in ATP, ADP and P_i concentration [37] as well as to effects due to competing reactions. The metabolic interrelationships at the triosephosphate level of glycolysis are illustrated by the scheme shown in Figure 1. In this scheme 1,3-DPGA is represented as the central metabolite whose fate is determined by several possible reactions. A reaction involving phosphocreatine kinase also occurs at this level, but not in RBC. Reactions iv and v represent the R. L. C. and involve an energy loss of 14 Kcal. *in toto*; 10 Kcal. in reaction iv and 4 Kcal. in reaction v [35]. Combinations of reactions i, iv and v as well as of reactions i and iii constitute self-regulating systems whereas a combination of reactions iii, iv and v simulates the action of ATPase. It is clear that predominance of the latter reaction sequence could cause leakage of P_i or inhibit the accumulation of 2,3-DPGA. Reaction v is stimulated by sodium bisulfite [39] and in some species also by Hg^{++} and 2-PGA [40], although

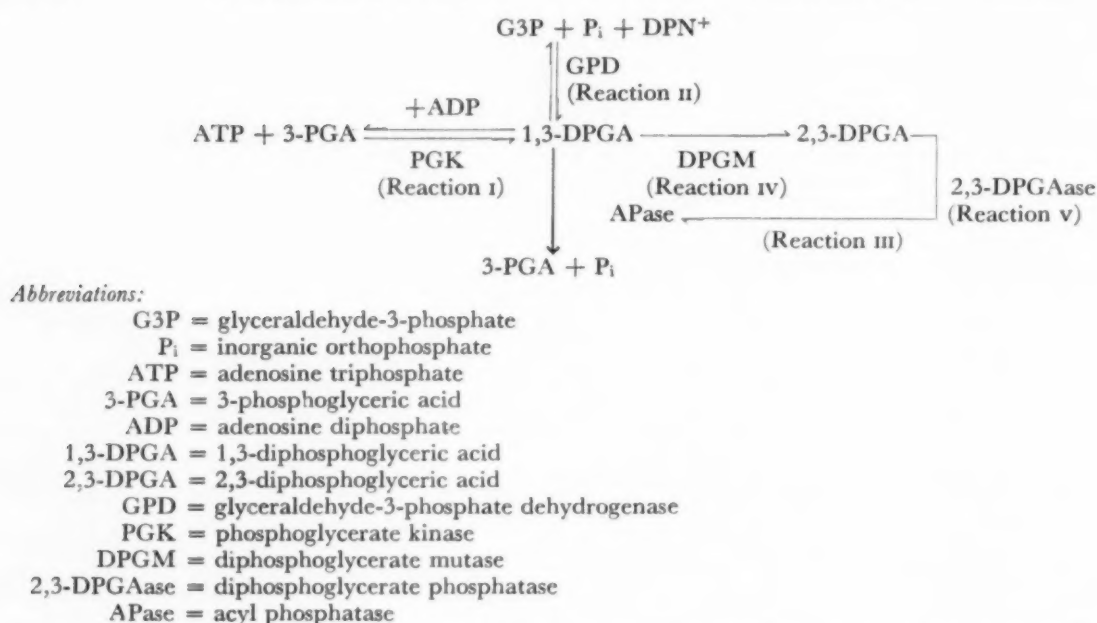


FIG. 1. The metabolic interrelationships between phosphoglyceric acids in human RBC.

Hg⁺⁺ fails to activate this enzyme in human RBC [38]. It is of interest in connection with the interrelationships at the triosephosphate level to note that thyroxine (5×10^{-6} M) inhibits reaction iv [40] to the extent of 90 per cent and also, although in higher concentrations, reaction iii [41,42]. It has been shown [43] that addition of triiodothyronine (TRIT) to intact RBC or hemolysates incubated with methylene blue increases the oxygen consumption of such systems as well as the oxidation of glucose to carbon dioxide. Although these findings were interpreted as reflecting a primary effect of TRIT on the oxidative pathway of glucose metabolism (OP), it is conceivable that the primary sites of action of TRIT are reactions iii and iv. (Fig. 1.) As mentioned before, thyroxine and related substances with hormonal activity have an inhibitory effect on reactions iii and v in concentrations of approximately the same magnitude found to be effective with TRIT. Inhibition of reactions iii and iv by TRIT would result in impairment of the energy-dissipating and regulatory mechanisms, with an increase in 1,3-DPG and in ATP. With ATP accumulating in intact RBC in the presence of adequate amounts of glucose and of methylene blue (MeB), G6P and TPNH formation as well as oxygen consumption might be expected to increase (as reported, [43]). In hemolysates fortified with G6P, inhibition of glycolysis would be associated with an increase in the steady-state

concentration of G6P and, consequently, with increased respiration. Thus the metabolic response *in vitro* of the RBC simulates the response to thyroid hormone and its derivatives observed in other cells of the body, in which an increased rate of oxidation of carbohydrate is paralleled by a decreased rate of phosphorylation. The operation of such a model system for thyroid hormone action is, of course, possible only in the presence of MeB in the RBC.

This proposed mechanism of the effect of TRIT on RBC metabolism serves well to illustrate the complexities encountered when two multienzyme systems compete with each other for common substrates. This point will be elucidated further by considering the interrelationships in the human RBC of the glycolysis pathway and other interlocking, ancillary pathways contributing to the catabolism of glucose. The relative physiological importance of each of these interrelated pathways is determined by the activity of its constitutive enzyme systems and the success with which the system can compete for components used in common with the other interlocking multienzyme systems.

The interrelationship between the glycolytic pathway and the OP is of special interest in human RBC inasmuch as the initial reaction of the OP is involved in the metabolism of GSSG (oxidized form of glutathione).

The two multienzyme systems interlock at several points at which they share the inter-

TABLE II
CHANGES IN THE ENZYMATIC PATTERN OF THE ERYTHROCYTE IN PATHOLOGICAL STATES

| Pathologic States | Over-all Glycolysis | Glycolytic Enzymes | Glucose-6-phosphate Dehydrogenase | Acetyl Cholinesterase | Arginase | Carbonic Anhydrase | Transketolase | Catalase | Glutathione Reductase | Phosphorylative Activity |
|---------------------------------------|---------------------|--------------------|-----------------------------------|-----------------------|----------|--------------------|---------------|----------|-----------------------|--------------------------|
| I. Hematologic disorders | | | | | | | | | | |
| A. Megaloblastic anemias | | | | | | | | | | |
| 1. Pernicious anemia | | | | | | | | | | |
| a. Untreated | | ↑(112) | | ↓(115) | ↑(113) | ↑(114) | | | | |
| b. Treated | | ↓(112) | | ↑(115) | | | | | | |
| 2. Megaloblastic anemias of pregnancy | | | | ↓(116) | | | | | | |
| B. Hemolytic anemias | | | | | | | | | | |
| 1. Hereditary | | | | | | | | | | |
| a. With spherocytosis | | | | | | | | | | ↓(91) |
| b. Without spherocytosis | ↓(117) | | (33,34)* | | | | | | | |
| c. Hemoglobinopathies | | | | | | | | | | |
| (α) Homozygous HbC disease | ↑(118) | | | | | | | | | |
| (β) Thalassemia major | | | | ↑(115) | ↑(117) | | | | | |
| d. Sick cell anemia | | | | | | | | | | ↑(132) |
| e. Sick cell trait | | | | | | | | | | ↑(132) |
| 2. Non-hereditary, PNH | | | | ↓(119) | | | | | | ↓(131,192) |
| 3. Acquired | | | | | | | | | | |
| a. Autoimmune | ↓(120) | | | | | | | | | ↓(94) |
| b. Induced | | | | | | | | | | |
| (α) Drugs | | | ↓(121) | | | | | ↓(125) | ↑(121) | |
| (β) Plumbism | | | | | ↓(122) | | | | | |
| (γ) Idiopathic thrombocytopenia | ↓(123) | | | | | | | | | |
| (δ) <i>Vicia fava</i> | | | ↓(121) | | | | | | | |
| (ε) Uremia | | | | | | | | | | ↓(124) |
| II. Hepatic diseases | | | | | | | | | | |
| A. Cirrhosis (Laennec's) | | ↑(126) | | | | | | | | ↓(126,127) |
| B. Hepatic coma | | ↑(126) | | | | | | | | ↓(126) |
| C. Hepatitis (acute viral) | | | ↑(126)† | | | | | | | |
| D. Obstructive jaundice | | | ↑(126)† | | | | | | | |
| III. Thiamine deficiency | | | | | | | | | | |
| Wernicke's disease | | | | | | | ↓(128) | | | |
| IV. Acidosis | ↓(129) | | | | | | | | | |
| | ↓(130) | | | | | | | | | |

NOTE: ↑ = denotes an increase in enzyme activity per RBC.

↓ = denotes a decrease in enzyme activity per RBC.

PNH = paroxysmal nocturnal hemoglobinuria.

* Enzyme absent.

† LD = Lactic dehydrogenase.

mediary metabolites G6P, F6P and G3P. The operation of the OP involves not only enzymes distinct from those of the glycolytic pathway but also glycolytic enzymes acting in retrograde fashion with respect to glycolysis. This participation of glycolytic enzymes in the OP ensures

the continuity of the cycle by making possible the regeneration of hexose phosphates. The relationships between the substrates common to both pathways and their respective enzymes are outlined in Table III.

The two pathways do not have in common:

TABLE III
INTERRELATIONSHIP BETWEEN GLYCOLYSIS AND THE PENTOSE PHOSPHATE OXIDATIVE PATHWAY
OF GLUCOSE CATABOLISM

| Substrate Common to Both Pathways | Enzymes | | | |
|--------------------------------------|--------------------|---|----------------------------|-----------------------|
| | Glycolytic Pathway | Cofactor Requirements | Oxidative Pathway | Cofactor Requirements |
| Glyceraldehyde-3-phosphate | GPD | P _i , DPN ⁺ , -SH | Transaldolase and aldolase | None |
| Fructose-6-phosphate | PFK | ATP, Mg ⁺⁺ | G6PI | None |
| Glucose-6-phosphate | G6PI | None | G6PD | TPN ⁺ |
| Fructose-1,6-diphosphate | Aldolase | None | FDPase * | Mg ⁺⁺ |

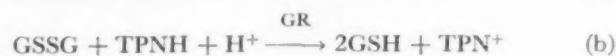
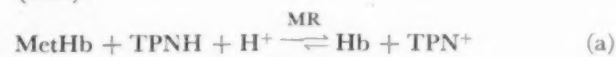
* The existence of a fructose-1,6-diphosphatase has not been demonstrated in the human RBC.

(a) requirements for ADP and P_i and thiamine pyrophosphate (TPP), or (b) the same pyridine nucleotide, since glycolysis is linked to DPN⁺ and the OP to TPN⁺. It is apparent from Table III that (a) G3P can be utilized in OP when DPN⁺-deficiency would prevent its participation in glycolysis, and (b) that G6P can be metabolized as a substrate of GPI in glycolysis whereas TPN⁺-deficiency would block its participation in the OP via G6PD. G6P may be expected to act primarily as a substrate of GPI since the limiting TPN⁺ concentration in the RBC restricts action of G6PD. Thus it may be concluded that glycolysis normally prevails in the human RBC and that the OP becomes functional only when an electron-transport system of high redox potential is present to ensure continued oxidation of TPNH. It should also be recalled that the absence of an active ATPase favors glycolysis since the PFK reaction remains without throttle. When the G6P concentration is limiting and the HK level low, PFK activity becomes the determining factor in the inhibition of the OP by glycolysis, in the presence of MeB. Thus, diversion of glycolysis into the OP requires not only highly active enzymatic systems in the OP but also conditions which would counterbalance the favorable position of glycolysis existing in the RBC, due to lack of active ATPase.

The Oxidative Pathway of Glucose Catabolism. The OP pathway does not appear to be operationally functional in spite of the presence of the required enzymes. The following circumstances account, in large measure, for the inability of the human RBC to oxidize glucose by way of the pentose phosphate cycle: (a) No electron

transport system exists in the human RBC through which molecular O₂ can be utilized for reoxidation of TPNH. (b) Although two systems can reoxidize TPNH, these systems do not regenerate TPN⁺ at a sufficiently rapid rate to ensure the continued activation of G6PD and 6PGD. (c) The oxidative decarboxylation of 6-phosphogluconate (6PG) to ribulose-5-phosphate (Ru5P) and carbon dioxide catalyzed by the dehydrogenase 6PGD requires TPN⁺. The total requirement for TPN⁺ in the OP is thereby increased. (d) Since a suitable electron transport system is missing in the human RBC, TPN is present predominantly in the reduced form TPNH. It appears doubtful, therefore, that sufficient TPN⁺ would be available for initiation of the OP at the dehydrogenase level.

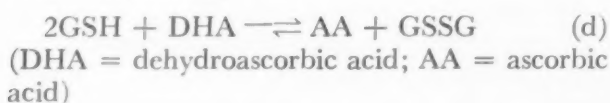
Dehydrogenases of the G6PD type (reaction c) couple readily with other oxidation-reduction systems utilizing alternatively the oxidized or reduced form of pyridine nucleotides, as shown for two such enzyme systems of importance to the RBC in reaction (a) methemoglobin reductase (MR) and (b) glutathione reductase (GR):



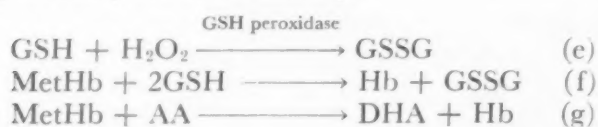
(where MetHb = methemoglobin, Hb = hemoglobin, 6PGL = 6-phosphogluconolactone).

In both reactions oxidizing TPNH (a and b) the equilibrium lies far in the direction of TPN⁺ whereas in the case of G6PD the equilibrium lies far in the direction of TPNH. For this reason the

reactions are virtually irreversible and the substrate cannot be regenerated by mere reversal of the reaction in the direction opposite to that indicated by the arrow. The supply of substrates available to the intact cell apparently is insufficient for continued operation of the OP in spite of possible contributions by other sources of enzymatic or non-enzymatic nature which could provide a "feed-back" mechanism by which the GSSG or MetHb pools would be replenished. One of these reactions is more likely to occur when the concentration of GSH is reduced (reaction d).



Other reactions of GSH and MetHb referred to previously are the following:



It is difficult to predict the position of the steady-state concentrations in these reactions, or to predict shifts in the equilibria accompanying metabolic changes in the cell. However, it is clear that GSSG and MetHb are not regenerated rapidly enough to maintain a TPN^+ concentration which would permit the formation of pentose phosphate, since the TPN^+ supply is limiting [45].

DRUG-INDUCED HEMOLYTIC ANEMIAS

A consideration of the biochemical aspects of drug-induced hemolytic anemias seems pertinent at this point since this type of anemia is associated with biochemical changes involving the coupled oxidation-reduction systems shown in equations (b) and (c). The cells of persons sensitive to certain drugs undergo hemolysis when exposed to the drug. Without such exposure, when no hemolytic manifestations are detectable, these RBC are characterized by: (a) G6PD activity $\frac{1}{4}$ to $\frac{1}{10}$ of that found in normal RBC, (b) a slight reduction in GSH content, and (c) no signs of increased osmotic fragility, decreased viability, immunological sensitivity, etc.

Recognition of drug-sensitive RBC has been made possible by applying the "GSH-stability test" [54], in which the effect of acetylphenylhydrazine (APH) on the GSH concentration of the RBC is measured. Drug-sensitive RBC ex-

hibit a marked decrease in GSH content, to about 10 per cent of the initial level. APH is used in this test system to tax the capacity of the cell to maintain a steady-state concentration of GSH and is known to produce two other effects of interest in this connection: (a) the formation of MetHb [55] and (b) the production of Heinz bodies [56,57].

In drug-sensitive RBC not treated with APH the concentration of GSH is 20 to 25 per cent [58] and that of G6PD is 10 to 25 per cent [59] below that of non-sensitive cells. Fegler [60] has demonstrated that ox RBC will not hemolyse unless the intracellular GSH concentration falls to about 40 per cent of the normal level. This suggests that the decreased GSH concentration in drug-sensitive cells should have no detectable effect (if the quantitative relationships in human RBC are similar to those in the ox RBC). The concentration of G6PD in the normal RBC probably exceeds that required for saturation by the amount of available substrate, i.e., only a fraction of the total amount of G6PD is in the activated state. The reduction of G6PD activity found in drug-sensitive cells would not be expected to affect the steady-state concentration of GSH except when there are extraordinary demands for TPNH, secondary to the need for a faster rate of GSSG reduction in order to replenish losses in GSH. In such circumstances the deficiency in G6PD becomes evident and is reflected in the resulting deficit in GSH. The data of Beutler et al. [67] concerning the balance of GSSG and GSH in drug-sensitive RBC treated with APH indicate a rapid decrease in GSH, together with a relatively slight decrease in GSSG, during a three-hour period of incubation. This relatively small reduction in GSSG concentration, compared with the large decrease in GSH concentration, indicates that production of GSH by way of the glutathione reductase (GR) reaction is retarded.* It is also possible that reoxidation of GSH to GSSG takes place, either due to heavy metal catalysis or by inter-

* It seems pertinent here to comment on the adequacy of the analytical methods used to determine GSH and GSSG concentrations, respectively. The small change in GSSG concentration concurrent with a marked change in GSH concentration may be the result of at least two factors: (a) the specificity of the method for determining GSSG is not high enough to exclude other compounds containing the disulfide group, i.e., cystine, bis-(γ -glutamyl-) cystine, —S—S— groups in globin of hemoglobin, etc. (b) the analytical sensitivity and accuracy of the analytical method for GSSG is inferior to that used for determination of GSH.

action with H_2O_2 , MetHb or other oxidizing agents, thus sustaining the initial GSSG concentration. An increased contribution by a biosynthetic pathway might also contribute in this respect [62]. In the test system of Beutler et al. [54] the appearance of Heinz bodies [56] may be considered evidence of partial destruction of hemoglobin. It is likely that an oxidation product of APH coordinates with hemoglobin [63] in a manner similar to that reported for phenylhydroxylamine [64], and that hemoglobin in this test system acts catalytically in the oxidation of GSH since it has been demonstrated that HbO_2 , MetHb and other related compounds catalyze the oxidation of GSH by ferricyanide [65]. Since Heinz-body formation has been associated with denaturation of hemoglobin [66], the reduction of intra- and intermolecular —S—S— bridges in the globin moiety by GSH may bring about a further decrease in the concentration of GSH and at the same time contribute to the GSSG pool.

The relationship of the G6PD system to the TPNH-linked MR system in drug-sensitive RBC is of interest in this connection. It is known that MetHb reacts with GSH non-enzymatically [67] to form GSSG + Hb. Therefore, the formation of appreciable amounts of MetHb may be expected to constitute a further drain on the GSH pool. Moreover, the deficiency in G6PD observed in RBC of patients with drug-induced anemia could bring about an accumulation of MetHb because of an insufficient supply of TPNH. However, the formation of MetHb does not seem to have been noted as a major clinical feature in drug-induced anemia, and it must therefore be assumed that the alternate DPNH-dependent MR [68,69] reduces MetHb at a sufficiently rapid rate to avoid accumulation of MetHb. * In the RBC of several members of an

Iranian family, G6PD was found to be entirely absent [33,34]. The affected members of this family had episodes of hemolytic activity with hyperbilirubinemia and increased osmotic fragility of their RBC. In one of the patients the TPNH-linked MR also was reduced and incubation of the RBC with phenylhydroxylamine produced no MetHb whereas normal RBC contained considerable amounts of MetHb under these conditions. It is possible that when G6PD is absent or deficient the ability to form MetHb is reduced, and thus accumulation of MetHb is avoided.

A correlation appears of interest in this context: the ability of some aromatic compounds, such as phenylhydrazine, to form Heinz bodies can be closely correlated with their ability to produce MetHb and to reduce GSH in drug-sensitive RBC at a rate considerably more rapid than the rate observed in non-sensitive RBC.

The formation of MetHb may be related to the production of Heinz bodies in that MetHb represents an intermediary stage in Heinz-body production. The relation between these two properties and the ability to reduce the concentration of GSH in drug-sensitive RBC remains unexplained.

There is ample evidence that sensitivity to drugs capable of inducing hemolytic anemias is a genetically controlled characteristic among certain groups of persons [70-76]. However, a deficiency in G6PD is not a specific attribute of drug-sensitive RBC, but also characterizes the hematologically normal, senescent RBC [28,78,79]. Three enzymes have been shown to decrease in activity in the aging RBC. The magnitude of decrease in enzymatic activity is greatest in the case of GPD (90 per cent) [28], as compared with 35 per cent for MR and 70 per cent for G6PD at the fiftieth day in the circulation (post-transfusion) [28]. All three enzymes known to decrease in activity with aging are pyridine nucleotide-linked, and the fall in enzyme activity seems to be synchronized with a diminution of the pyridine nucleotide content [28]. Furthermore, it has been reported that the —SH content decreases whereas the oxygen consumption increases in senescent RBC [28]. It remains to be determined in what way these changes are related to the destruction of senescent RBC and by what mechanism the activity of enzymes is reduced in a progressive manner with advancing age of the cell. Similarly, the mechanism by which deficiency in G6PD con-

* Another plausible explanation might be advanced to account for the apparent absence of MetHb [67] *in vitro* in APH-treated drug-sensitive RBC and *in vivo* in RBC during a hemolytic crisis following administration of the sensitizing drug. If reaction (f) mentioned previously were to operate to a significant extent, MetHb, even if formed in significantly large quantity, would escape detection due to rapid reversion to HbO_2 ; only the disappearance of GSH would be observed. Since two molecules of GSH are required for the formation of one molecule of GSSG, the effect of the operation of reaction (f) on GSSG concentration would be smaller than the effect on GSH concentration. The latter point may, in part, explain the divergency in regard to changes in GSSG and GSH concentration, respectively, observed by Beutler et al. [67].

tributes to the drug-induced hemolytic process remains to be elucidated.

The degree of G6PD deficiency of the RBC appears to be related to the severity and reversibility of the hemolytic disease. Thus the hemolytic tendency exhibited by drug-sensitive RBC which are deficient in but not wholly devoid of G6PD may be differentiated from the hemolytic state associated with complete absence of G6PD in the RBC of the affected members of an Iranian family [33,34], as follows: (a) The hemolytic process can be induced by the sensitizing drug only under *in vivo* conditions whereas RBC devoid of G6PD hemolyse *in vitro* also. (b) The drug-induced hemolytic process is limited to a maximal drug response which can not be exceeded by further administration of the sensitizing agent. (c) The drug-induced hemolytic state is limited in duration and is abolished when most of the senescent RBC, which are the most G6PD-deficient group in the circulating RBC population, have been destroyed. The hemolytic process stimulates erythropoiesis whereupon a large number of young RBC of relatively high G6PD-content enter the circulation, thus contributing cells relatively resistant to hemolysis. When the average activity of G6PD in the cell population is 10 per cent to 40 per cent of the activity of a normal cell population, the hemolytic tendency seems readily reversible because the destructive process is limited largely to the senescent RBC, which are most deficient in G6PD activity.

Thus the hemolytic crises observed by Löhr and collaborators [33,34] in the affected members of one family were considerably more severe, occurred spontaneously without provocation by a sensitizing agent, and were associated with marked hyperbilirubinemia. RBC from such patients contain no G6PD, hemolyse *in vitro*, and exhibit abnormal osmotic fragility.

The coupling of the dehydrogenase system with GR may serve to extend further the control over survival exerted by G6PD, by maintaining GSH concentrations required for preservation of the cellular integrity [80]. Despite competition of glycolytic enzymes for G6P and despite the inactivity of the OP, the RBC contains the necessary enzymes for the operation of this pathway [81,82]. Certain parts of the pathway could therefore be activated if the cell were supplied with suitable substrates entering the OP at a level at which TPN⁺ would not be required. The intact RBC is impermeable to most phos-

phorylated substances, but activation of OP is possible by supplying purine nucleosides, either to hemolyzate [83] or to the intact RBC [84-89]. In either system, purine nucleosides are transformed to R1P and the nitrogenous base, as a result of phosphorylytic cleavage catalyzed by the enzyme purine nucleoside phosphorylase (PNP) in the presence of Mg⁺⁺ and P_i. Purine nucleosides are utilized by intact RBC after prolonged low-temperature storage to regenerate ATP when glucose can no longer serve this purpose [84]. Similar findings have been reported after short-term storage at room temperature [3]. The phosphorylytic cleavage of the N-glycosidic linkage in purine nucleosides is an exergonic reaction, and the energy liberated as a result of cleavage of the N—C bond could be utilized by the RBC for maintenance of cation transport functions. Human RBC contain phosphoribomutase [90], which transforms R5P into R1P. The presence of phosphoribose isomerase (PRI) ensures conversion of R5P to ribulose-5-phosphate (Ru5P) and, by the action of an epimerase, also conversion to xylulose-5-phosphate (Xu5P). In the subsequent reactions, which are catalyzed alternately by transketolase (TK) activated by thiamine pyrophosphate (TPP) and transaldolase (TA1), the following over-all conversion occurs:



In this way, the three intermediates common to glycolysis and also OP, F6P, G6P and G3P, can be regenerated.

It is conceivable that inosine and other suitable purine nucleosides bring about the regeneration of ATP in ATP-depleted cells by supplying G3P to initiate the formation of 1,3-DPGA. If only small quantities of ADP are available, partial activation of the OP, by supplying a potential source of pentose phosphate, may prolong the "life" of the cell. There are two aspects of this reaction which are beneficial insofar as survival of the cell is concerned: (a) the metabolic bypass via a portion of the OP requires no adenine nucleotides, i.e., has an "ATP-sparing" action, and (b) a substrate is provided which promote formation of 1,3-DPGA. However, if the ADP content of the depleted RBC has decreased to a level insufficient for activation of PGK, this pathway will be of no avail in initiating ATP-regeneration.

Purine nucleosides may be considered "pre-formed" carbohydrates incorporated into a

vehicle palatable to the RBC and having survival value under conditions where glucose can no longer be utilized. However, purine nucleosides cannot sustain the operation of a metabolic cycle since pentose phosphate regeneration can take place only by way of the oxidative portion of the alternate pathway of glucose catabolism. Purine nucleosides, therefore, are ineffective when all of the added material has been phosphorylated, but as a result of this phosphorylolytic process purine nucleosides stimulate ATP-regeneration.

HEREDITARY SPHEROCYTOSIS

In hereditary spherocytosis (HS) the flux of P_i into the GPD reaction is considerably slower than that normally observed [97]. The extent of labelling of ATP by P_i^{32} added to the extracellular medium is reduced whereas the initial labelling of intracellular P_i is markedly increased as compared with that of ATP. The exchange of P_i across the cell membrane and incorporation of P_i^{32} into phosphorylated glycolytic intermediates of RBC from patients with HS are inhibited by F^- concentrations ineffective in hematologically normal cells [92]. It may be speculated on the basis of these observations that the rate of regeneration of ATP is reduced in HS cells, and that an enzyme system which is inhibited by F^- may be deficient in such cells. The nature of the enzyme involved, if indeed there be such, is not known. It has been shown that ATPase is not decreased in HS cells [93] and it seems unlikely that hexokinase and phosphofructokinase are deficient since these enzymes are not inhibited by F^- in concentrations affecting P_i -incorporation and P_i exchange in RBC from patients with HS. Since enolase is characteristically sensitive to relatively low concentrations of F^- of the magnitude found to affect HS cells, this may be the enzyme present in limiting concentration in HS cells. The validity of such a hypothesis must await experimental test.

The normal distribution pattern of P^{32} activity and the normal time course of the incorporation of P_i^{32} into the intermediates of glycolysis can be restored *in vitro* by incubating HS cells in a medium containing purine ribosides. However, RBC from a relatively small group of patients with HS, including several members of the same family, failed to respond to purine ribosides in the way mentioned [97]. The metabolic anomaly observed in HS cells is presumed to be an intrinsic characteristic of the cell because it

persists after splenectomy. Other types of hemolytic anemia do not exhibit the combination of metabolic changes observed in the RBC of patients with HS, although they have been found to show some, but not all, of the metabolic changes of HS cells [94]. The RBC of patients with congenital, non-spherocytic hemolytic anemia contain 2,3-DPGA in higher than normal concentration [95], perhaps due to a defect in 2,3-DPGAase (reaction v, Fig. 1).

THE URIDYL TRANSFERASE PATHWAY

Another ancillary pathway for the metabolism of glucose potentially available in human RBC consists of a series of reversible reactions which function in the transfer of uridyl moieties between hexosephosphates. This "transferase pathway" (TP) may be of minor importance to glucose metabolism in the normal human RBC, except for its possible role in the biosynthesis of glycogen. However, the TP of the RBC has been of considerable importance in investigations leading to recognition of the biochemical defect in galactosemia [96].

In the human RBC the TP also depends upon the key metabolite G6P for its initiating substrate (as did the OP already discussed). The enzyme PGM catalyzed the conversion of G6P to glucose-1-phosphate (G1P) in the presence of catalytic amounts of glucose-1,6-diphosphate (GDP). The important intermediate of the TP is uridine diphosphoglucose (UDPG) which is formed when G1P reacts with uridine triphosphate (UTP) in the presence of UDPG pyrophosphorylase. UDPG participates in two important reactions: (a) epimerization to UDP galactose (UDPGal) as a result of the action of uridine diphosphogalactose-4-epimerase and DPN^+ , or (b) conversion to UDPGal and G1P, catalyzed by galactose-1-phosphate uridyl transferase. Congenital absence of the latter enzyme is recognized as the biochemical lesion of galactosemia [96]. Galactose-1-phosphate (gal-1-P) has been reported [97] to inhibit PGM. Since gal-1P accumulates in RBC of patients with galactosemia, the concentration of G1P, and also of glycogen, if present, might be expected to be low in such RBC. Glycogen occurs in human RBC [98] and it seems possible that the RBC may synthesize this polysaccharide from UDPG by the mechanism proposed by Leloir [99]. Whether this synthetic reaction can take place depends upon the availability of UTP, which has been found in rabbit RBC [100].

SUMMARY

The human erythrocyte engages solely in a glycolytic type of metabolic activity, a metabolic specialization which may be viewed as an expression of adaptive efforts directed toward survival in an environment rich in glucose, and in the face of a "handicap" due to the lack of a nucleus. Since relatively few biosynthetic reactions occur in erythrocytes, a specialized metabolic pathway, the Rapoport-Luebering cycle, has evolved in the human erythrocyte which provides a means of dissipating the excess of energy-rich phosphate so as to ensure continued glycolytic activity. Although anaerobic glycolysis constitutes the only important pathway for the catabolism of glucose in the human erythrocyte under normal physiological conditions, the cell is potentially capable of catabolizing glucose oxidatively as well as of metabolizing glucose by means of the uridyl transferase reaction sequence.

The human erythrocyte is enzymatically equipped to oxidize glucose by way of the pentose phosphate metabolic cycle, but this alternate pathway of glucose catabolism can be activated only if the normally missing metabolic link to molecular oxygen is supplied. Partial activation can be achieved in the non-oxidative portion of the pentose phosphate cycle by supplying a suitable substrate.

The glycolipoprotein-containing envelope of the human erythrocyte appears to perform an important metabolic function in regulating the flux of ions in and out of the cell and in controlling the flow of glucose and phosphate, both of which are essential metabolites for the human erythrocyte.

Metabolic aberrations have been found in a variety of pathologic conditions, but only a few of these have been elucidated sufficiently to permit exact definition of the aberration. Most firmly established in the latter category are the metabolic aberrations found in drug-induced hemolytic anemia, in galactosemia and in hereditary spherocytosis.

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REFERENCES

1. KLINGHOFFER, K. A. The distribution of glucose between cells and serum: further experiments with high concentrations of glucose. *Am. J. Physiol.*, 130: 89, 1940.
2. TABECHIAN, H., ALTMAN, K. I., YOUNG, L. E. and BARTLETT, J. Effect of insulin on phosphorus metabolism in normal red blood cells and in red blood cells of patients with schizophrenia and depressive psychosis. *J. Clin. Invest.* To be published.
3. PRANKERD, T. A. J. and ALTMAN, K. I. A study of the metabolism of phosphorus in mammalian red cells. *Biochem. J.*, 58: 622, 1954.
4. BARTLETT, G. R. Organization of red cell glycolytic enzymes: cell coat phosphorus transfer. *Ann. New York Acad. Sc.*, 75: 110, 1958.
5. MORRISON, W. L. and NEURATH, H. Proteolytic enzymes of the formed elements of human blood. I. Erythrocytes. *J. Biol. Chem.*, 200: 39, 1953.
6. ADAMS, E., MCFADDEN, M. and SMITH, E. L. Peptidases of erythrocytes. I. Distribution in man and other species. *J. Biol. Chem.*, 198: 663, 1952.
7. ADAMS, E. and SMITH, E. L. Peptidases of erythrocytes. II. Isolation and properties of prolidase. *J. Biol. Chem.*, 198: 671, 1952.
8. ADAMS, E., DAVIS, N. C. and SMITH, E. L. Peptidases of erythrocytes. III. Tripeptidase. *J. Biol. Chem.*, 199: 845, 1952.
9. COLLIER, H. B. and SOLVONUK, P. F. Acylation reactions in the presence of acetylcholinesterase of human erythrocytes. *Biochim. et biophys. acta*, 16: 583, 1955.
10. ZITTLE, C. A., DELLAMONICA, E. S. and CUSTER, J. H. Purification of human red cell acetylcholinesterase. *Arch. Biochem. & Biophys.*, 48: 43, 1954.
11. CLARKSON, E. M. and MAIZELS, M. Distribution of phosphatase in human erythrocytes. *J. Physiol.*, 116: 112, 1952.
12. CAFFREY, R. W., TREMBLAY, R., GABRIO, B. W. and HUENNEKENS, F. M. Erythrocyte metabolism. II. Adenosinetriphosphatase. *J. Biol. Chem.*, 223: 1, 1956.
13. HERBERT, E. A study of the liberation of orthophosphate from adenosine triphosphate by the stromata of human erythrocytes. *J. Cell. Comp. Physiol.*, 47: 11, 1956.
14. SZÉKELY, M., MANYAI, S. and STRAUB, F. B. Die Wirkung der Hämolyse auf den Stoffwechsel der roten Blutkörperchen beim Menschen. *Acta physiol. Hung.*, 4: 31, 1953.
15. GÁRDOS, G. Akkumulation der Kaliumionen durch menschliche Blutkörperchen. *Acta physiol. Hung.*, 6: 191, 1954.
16. GARZÓ, T., ULLMANN, A. and STRAUB, F. B. Die ATP-ase der roten Blutkörperchen. *Acta physiol. Hung.*, 3: 513, 1952.
17. ALIVISATOS, S. G. A., KASHKET, S. and DENSTEDT, O. F. The metabolism of the erythrocyte. IX. Diphosphopyridine nucleotidase of erythrocytes. *Canad. J. Biochem. & Physiol.*, 34: 46, 1956.
18. MALKIN, A. and DENSTEDT, O. F. The metabolism of the erythrocyte. XII. Diphosphopyridine nucleotide nucleosidase of the rabbit erythrocyte. *Canad. J. Biochem. & Physiol.*, 34: 141, 1956.
19. GROSSMAN, L. and KAPLAN, N. O. A possible enzymatic role of ergothioneine. *J. Am. Chem. Soc.*, 78: 4175, 1956.

20. GOODMAN, J. R., MARRONE, L. H. and SQUIRE, M. C. Effect of *in vivo* inhibition of cholinesterase on potassium diffusion from the human red cell. *Am. J. Physiol.*, 180: 118, 1955.
21. LINDVIG, P. E., GREIG, M. E. and PETERSON, S. W. Studies on permeability. v. The effects of acetylcholine and physostigmine on the permeability of human erythrocytes to sodium and potassium. *Arch. Biochem.*, 30: 241, 1951.
22. GREIG, M. E., FAULKNER, J. S. and MAYBERRY, T. C. Studies on permeability. ix. Replacement of potassium in erythrocytes during cholinesterase activity. *Arch. Biochem.*, 43: 39, 1953.
23. GOETZE, E. and RAPOPORT, S. Das Kathepsin der Kaninchenerythrocyten und seine Veränderungen bei der Zellreifung. *Biochem. Ztschr.*, 326: 53, 1954.
24. GÁRDOS, G. and STRAUB, F. B. Über die Rolle der Adenosine-triphosphorsäure (ATP) in der K-Permeabilität der menschlichen roten Blutkörperchen. *Acta physiol. Hung.*, 12: 1, 1957.
25. ROTHSTEIN, A. Enzyme systems of the cell surface involved in the uptake of sugars by yeast. *Symp. Soc. Exper. Biol.*, 8: 165, 1954.
26. PRANKERD, T. A. J. and ALTMAN, K. I. The effect of adenosine on the phosphate exchange in mammalian red blood cells. *Biochim. et biophys. acta.*, 15: 158, 1954.
27. ALLISON, A. C. and BURN, G. P. Enzyme activity as a function of age in the human erythrocyte. *Brit. J. Haematol.*, 1: 291, 1955.
28. LÖHR, G. W., WALLER, H. D., KARGES, O., SCHLEGEL, B. and MÜLLER, A. A. Zur Biochemie der Alterung menschlicher Erythrocyten. *Klin. Wchnschr.*, 36: 1008, 1958.
29. MARKS, P. A., JOHNSON, A. B. and HIRSCHBERG, E. Effect of age on the enzyme activity in erythrocytes. *Proc. Nat. Acad. Sc.*, 44: 529, 1958.
30. BURNET, F. M. and EDNEY, M. Influence of ions on the interaction of influenza virus and cellular receptors or soluble inhibitors of haemagglutination. *Australian J. Exper. Biol. & M. Sc.*, 30: 105, 1952.
31. PULSFORD, M. F. Factors affecting the lysis of erythrocytes treated with staphylococcal β toxin. *Australian J. Exper. Biol. & M. Sc.*, 32: 347, 1954.
32. LÖHR, G. W., WALLER, H. D. and KARGES, O. Quantitative Fermentbestimmungen in roten Blutzellen. *Klin. Wchnschr.*, 35: 871, 1957.
33. WALLER, H. D., LÖHR, G. W. and TABATABAI, M. Hämolyse und Fehlen von Glucose-6-Phosphatdehydrogenase in roten Blutzellen. *Klin. Wchnschr.*, 35: 1022, 1957.
34. LÖHR, G. W. and WALLER, H. D. Hämolytische Erythropathie durch Fehlen von Glucose-6-Phosphatdehydrogenase in roten Blutzellen als dominant vererbliche Krankheit. *Klin. Wchnschr.*, 36: 865, 1958.
35. RAPOPORT, S. and LUEBERING, J. The formation of 2,3-diphosphoglycerate in rabbit erythrocytes: the existence of a diphosphoglycerate mutase. *J. Biol. Chem.*, 183: 507, 1950.
36. RAPOPORT, S. and LUEBERING, J. Glycerate-2,3-diphosphatase. *J. Biol. Chem.*, 189: 683, 1951.
37. RAPOPORT, S. and NIERADT, C. Inwiefern verläuft die Glykolyse im Säugetier-erythrocyten über 2,3-Diphosphoglycerinsäure? Über eine Variante des glykolytischen Cyclus auf dem Niveau der Phosphoglycerinsäuren. *Biochem. Ztschr.*, 326: 231, 1955.
38. JOYCE, B. K. and GRISOLIA, S. The purification and properties of muscle diphosphoglycerate mutase. *J. Biol. Chem.*, 234: 1330, 1959.
39. MÁNYAI, S. and VÁRADY, Z. Elektive Spaltung der 2,3-Diphosphoglycerinsäure in Erythrozyten. *Biochim. et biophys. acta*, 20: 594, 1956.
40. RAPOPORT, S., LUEBERING, J. and WAGNER, R. H. Über die Quecksilber-Aktivierung der Glycerinsäure-2,3-diphosphatase. *Ztschr. physiol. Chem.*, 302: 105, 1955.
41. HARARY, I. The effect *in vivo* of thyroxine on acylphosphatase of rat liver and muscle. *Biochim. et biophys. acta*, 29: 647, 1958.
42. HARARY, I. The *in vitro* inhibition of acetylphosphatase by thyroxine. *Biochim. et biophys. acta*, 25: 193, 1957.
43. NECHELES, T. and BEUTLER, E. The effect of triiodothyronine on the oxidative metabolism of erythrocytes. i. Cellular studies. *J. Clin. Invest.*, 38: 788, 1959.
44. BLANCHARD, M. C., BROWNSTONE, S. and WILLIAMS, H. R. Decreased phosphofructokinase activity in preserved blood. *Am. J. Physiol.*, 183: 95, 1955.
45. COURT, D. and RACKER, E. The oxidative pentose phosphate cycle. v. Oxidation of glucose-6-phosphate in a reconstructed system of the oxidative pentose phosphate cycle. *Arch. Biochem.*, 83: 195, 1959.
46. BARTLETT, G. R. Human red cell glycolytic intermediates. *J. Biol. Chem.*, 234: 449, 1959.
47. BISHOP, C., RANKINE, D. M. and TALBOTT, J. H. The nucleotides in normal human blood. *J. Biol. Chem.*, 234: 1233, 1959.
48. BERNSTEIN, R. E. Rates of glycolysis in human red cells in relation to energy requirements for cation transport. *Nature, London*, 172: 911, 1953.
49. RALL, T. W. and LEHNINGER, A. L. Glutathione reductase of animal tissues. *J. Biol. Chem.*, 194: 119, 1952.
50. COLLIER, H. B. and McRAE, S. C. The amperometric determination of glutathione reductase activity in human erythrocytes. *Canad. J. Biochem. & Physiol.*, 33: 404, 1955.
51. KIESE, M. and SCHWARTZKOPFF, W. Die Reduktion des Hämoglobins. iii. Mitteilung. Reduktion des Hämoglobins und Stoffwechsel in roten Zellen. *Arch. exper. Path. u. Pharmacol.*, 204: 267, 1947.
52. KIESE, M., SCHNEIDER, C. and WALLER, H. D. Hämoglobinreduktase. *Arch. exper. Path. u. Pharmacol.*, 231: 158, 1957.
53. HUENNEKENS, F. M., CAFFREY, R. W. and GABRIO, B. W. The electron transport sequence of methemoglobin reductase. *Ann. New York Acad. Sc.*, 75: 167, 1958.
54. BEUTLER, E., ROBSON, M. J. and BUTTENWIESER, E. The glutathione instability of drug sensitive red cells. *J. Lab. & Clin. Med.*, 49: 84, 1957.
55. BEAVEN, G. H. and WHITE, J. C. Oxidation of phenylhydrazines in the presence of oxyhaemoglobin and the origin of Heinz bodies in erythrocytes. *Nature, London*, 173: 389, 1954.
56. BEUTLER, E., DERN, R. J. and ALVING, A. S. The

- hemolytic effect of primaquine. III. A study of primaquine-sensitive erythrocytes. *J. Lab. & Clin. Med.*, 45: 40, 1955.
57. ROZSA, G. and SPICER, S. S. The nature of Heinz bodies. *Nature, London*, 171: 84, 1953.
 58. BEUTLER, E., DERN, R. J., FLANAGAN, C. L. and ALVING, A. S. The hemolytic effect of primaquine. VII. Biochemical studies of drug-sensitive erythrocytes. *J. Lab. & Clin. Med.*, 45: 286, 1955.
 59. GROSS, R. T., HURWITZ, R. E. and MARKS, P. A. An hereditary enzymatic defect in erythrocyte metabolism: glucose-6-phosphate dehydrogenase deficiency. *J. Clin. Invest.*, 37: 1176, 1958.
 60. FEGLER, G. Relationship between reduced glutathione content and spontaneous haemolysis in shed blood. *Nature, London*, 170: 624, 1952.
 61. BEUTLER, E., ROBSON, M. and BUTTENWIESER, E. The mechanism of glutathione destruction and protection in drug-sensitive and non-sensitive erythrocytes. *In vitro* studies. *J. Clin. Invest.*, 36: 617, 1957.
 62. DIMANT, E., LANDSBERG, E. and LONDON, I. M. The metabolic behavior of reduced glutathione in human and avian erythrocytes. *J. Biol. Chem.*, 213: 769, 1955.
 63. BRENNER, S. and ALLISON, A. C. Catalase inhibition: a possible mechanism for the production of Heinz bodies in erythrocytes. *Experientia*, 11: 381, 1953.
 64. BRINKMANN, F. and KIESE, M. Oxydation von Hämoglobin und Muskelhämoglobin durch Phenylhydroxylamin und Sauerstoff; Abhängigkeit vom Sauerstoffdruck. *Biochem. Ztschr.*, 326: 218, 1955.
 65. GIBSON, Q. H. The reduction of methaemoglobin in red blood cells and studies on the cause of idiopathic methaemoglobinaemia. *Biochem. J.*, 42: 13, 1948.
 66. FERTMAN, M. H. and FERTMAN, M. B. Toxic anemias and Heinz bodies. *Medicine*, 34: 131, 1955.
 67. EGGLETON, P. and FEGLER, G. Reduced glutathione and spontaneous methaemoglobin formation in haemolysates. *Quart. J. Exper. Physiol.*, 37: 163, 1952.
 68. KIESE, M. Die Reduktion des Hämoglobins. *Biochem. Ztschr.*, 316: 264, 1944.
 69. KIESE, M. Die Reduktion des Hämoglobins. v. Mitteilung. *Arch. exper. Path. u. Pharmacol.*, 204: 451, 1947.
 70. DERN, R. J., WEINSTEIN, I. M., LEROY, G. V., TALMAGE, D. W. and ALVING, A. S. The hemolytic effect of primaquine. I. The localization of the drug-induced hemolytic defect in primaquine-sensitive individuals. *J. Lab. & Clin. Med.*, 43: 303, 1954.
 71. SZEINBERG, A., SHEBA, C. and ADAM, A. Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes. *Blood*, 13: 1043, 1958.
 72. CHILDS, B., ZINKHAM, W., BROWNE, E. A., KIMBRO, E. L. and TORBERT, J. V. A genetic study of a defect in glutathione metabolism of the erythrocyte. *Bull. Johns Hopkins Hosp.*, 102: 21, 1958.
 73. LARIZZA, P., BRUNETTI, P., GRIGNANI, F. and VENTURA, S. L'individualita bio-enzimatica dell'eritrocito "fabico" sobre alcune anomalie biochimiche ed enzimatiche delle emazie nei pazienti affetti da favismo e nei loro familiari. *Haematologica*, 43: 205, 1958.
 74. SANSONE, G. and SEGNI, G. L'instabilita del glutathione ematico (GSH) nel favismo utilizzazione di un test selettivo. Introduzione al problema genetico. *Boll. Soc. ital. biol. sper.*, 33: 1057, 1957.
 75. SZEINBERG, A., ASHER, Y. and SHEBA, C. Studies on glutathione stability in erythrocytes of cases with past history of favism or sulfa-drug-induced hemolysis. *Blood*, 13: 348, 1958.
 76. ZINKHAM, W. H. and CHILDS, B. A defect of glutathione metabolism in erythrocytes from patients with a naphthalene induced hemolytic anemia. *Pediatrics*, 22: 461, 1958.
 77. MARKS, P. A. and GROSS, R. T. Drug-induced hemolytic anemias and congenital galactosemia. *Bull. New York Acad. Med.*, 35: 433, 1959.
 78. MARKS, P. A., JOHNSON, A. B., HIRSCHBERG, E. and BANKS, J. Studies on the mechanism of aging of human red blood cells. *Ann. New York Acad. Sci.*, 75: 95, 1958.
 79. MÁNYAI, S. Stoffwechseleränderungen im Laufe der Entwicklung der roten Blutkörperchen. I. Untersuchungen mit dem Blut verschieden alter Ratten. *Acta physiol. Hung.*, 5: 19, 1954.
 80. BENESCH, R. E. and BENESCH, R. Relation between erythrocyte integrity and sulphydryl groups. *Arch. Biochem. & Biophys.*, 48: 38, 1954.
 81. BRUNS, F. H., NOLTMANN, E. and VAHLHAUS, E. Über den Stoffwechsel von Ribose-5-phosphat in Hämolysaten. I. Aktivitätsmessung und Eigenschaften der Phosphoribose-isomerase. II. Der Pentosephosphat-Cyclus in roten Blutzellen. *Biochem. Ztschr.*, 330: 483, 1958.
 82. BRUNS, F. H., DÜNWALD, E. and NOLTMANN, E. Über den Stoffwechsel von Ribose-5-phosphat in Hämolysaten. III. Quantitative Bestimmung von Sedoheptulose-7-phosphat und einige Eigenschaften der Transketolase der Erythrocyten und des Bluteserums. *Biochem. Ztschr.*, 330: 497, 1958.
 83. DISCHE, Z. Synthesis of hexosomono- and diphosphate from adenosine and ribose-5-phosphate in human blood. In: *Phosphorus Metabolism*, vol. 1, p. 171. Edited by McElroy, W. D. and Glass, B. Baltimore, 1951. Johns Hopkins Press.
 84. GABRIO, B. W., HENNESSEY, M., THOMASSON, J. and FINCH, C. A. Erythrocyte preservation. IV. *In vitro* reversibility of the storage lesion. *J. Biol. Chem.*, 215: 357, 1955.
 85. GABRIO, B. W., DONOHUE, D. M. and FINCH, C. A. Erythrocyte preservation. V. Relationship between chemical changes and viability of stored blood treated with adenosine. *J. Clin. Invest.*, 34: 1509, 1955.
 86. DONOHUE, D. M., FINCH, C. A. and GABRIO, B. W. Erythrocyte preservation. VI. The storage of blood with purine nucleosides. *J. Clin. Invest.*, 35: 562, 1956.
 87. GABRIO, B. W., FINCH, C. A. and HUENNEKENS, F. M. Erythrocyte preservation: a topic in molecular biochemistry. *Blood*, 11: 103, 1956.
 88. HUENNEKENS, F. M., LIU, L., MYERS, H. A. P. and GABRIO, B. W. Erythrocyte metabolism. III.

- Oxidation of glucose. *J. Biol. Chem.*, 227: 253, 1957.
89. YOUNG, L. E., IZZO, M. J., ALTMAN, K. I. and SWISHER, S. N. Studies on spontaneous *in vitro* autohemolysis in hemolytic disorders. *Blood*, 11: 977, 1956.
 90. GUARINO, A. J. and SABLE, H. Z. Studies on phosphomutases. II. Phosphoribomutase and phosphoglucumutase. *J. Biol. Chem.*, 215: 515, 1955.
 91. PRANKERD, T. A. J., ALTMAN, K. I. and YOUNG, L. E. Abnormalities of carbohydrate metabolism of red cells in hereditary spherocytosis. *J. Clin. Invest.*, 34: 1268, 1955.
 92. ALTMAN, K. I., TABECHIAN, H. and YOUNG, L. E. Some aspects of the metabolism of red blood cells from patients with hemolytic anemias. *Ann. New York Acad. Sci.* 75: 142, 1958.
 93. PRANKERD, T. A. J. Personal communication.
 94. PRANKERD, T. A. J. Red-cell structure and metabolism in haemolytic anaemia. *Brit. M. Bull.*, 15: 54, 1959.
 95. MOTULSKY, A. G., GABRIO, B. W., BURCKHARDT, J. and FINCH, C. A. Erythrocyte carbohydrate metabolism in hereditary hemolytic anemias. *Am. J. Med.*, 19: 291, 1955.
 96. KALCKAR, H. M. and MAXWELL, E. S. Biosynthesis and metabolic function of uridine diphosphoglucose in mammalian organisms and its relevance to certain inborn errors. *Physiol. Rev.*, 38: 77, 1958.
 97. SIDBURY, J. B., JR. The enzymatic lesions in galactosemia. *J. Clin. Invest.*, 36: 929, 1957.
 98. BARTLETT, G. R. Personal communication.
 99. LELOIR, L. F. and CARDINI, C. E. Biosynthesis of glycogen from uridine diphosphate glucose. *J. Am. Chem. Soc.*, 79: 6340, 1957.
 100. SCHWEIGER, H. G. and RAPOPORT, S. Der N-Stoffwechsel bei der Erythrocytenreifung: Die N-Bilanz unter endogenen Bedingungen. *Ztschr. physiol. Chem.*, 313: 97, 1958.
 101. BRUNS, F. H. and VAHLHAUS, E. Phosphoriboisomerase und Phosphohexoisomerase in Serum, roten Blutzellen und Geweben. *Naturwiss.*, 43: 180, 1956.
 102. BARTLETT, G. R. and MARLOW, A. A. Enzyme systems in the red blood cell. *Bull. Scripps Metabol. Clin.*, vol. 2, 1951.
 103. BRUNS, F. Über die Aldolase der Erythrocyten. *Biochem. Ztschr.*, 325: 429, 1954.
 104. BEHRENDT, H. Chemistry of Erythrocytes. Springfield, Ill., 1957. Charles C Thomas.
 105. TSUBOI, K. K. and HUDSON, P. B. Enzymes of the human erythrocyte. I. Purine nucleoside phosphorylase; isolation procedure. *J. Biol. Chem.*, 224: 879, 1957.
 106. TSUBOI, K. K. and HUDSON, P. B. Acid Phosphatase. I. Human red cell phosphomonoesterase; general properties. *Arch. Biochem. & Biophys.*, 43: 339, 1953.
 107. NOLTMANN, E. and BRUNS, F. H. Über die Phosphoglucumutase der Erythrocyten und des Serums. *Ztschr. physiol. Chem.*, 313: 194, 1958.
 108. TATIBANA, M., NAKAO, M. and YOSHIKAWA, H. Adenylate kinase in human erythrocytes. *J. Biochem. (Jap.)*, 45: 1037, 1958.
 109. KASHKET, S., RUBINSTEIN, D. and DENSTEDT, O. F. Studies on the preservation of blood. v. The influence of the hydrogen ion concentration on certain changes in blood during storage. *Canad. J. Biochem. & Physiol.*, 35: 827, 1957.
 110. MARKS, P. A. Red cell glucose-6-phosphate and 6-phosphogluconic dehydrogenase and nucleoside phosphorylase. *Science*, 127: 1338, 1958.
 111. DRABKIN, D. L. and SCHMIDT, C. F. Spectrophotometric studies. XII. Observation of circulating blood *in vivo* and the direct determination of the saturation of hemoglobin in arterial blood. *J. Biol. Chem.*, 157: 69, 1945.
 112. BOCK, H. E. and WALLER, H. D. Besonderheiten im Fermentgehalt von Megalocyten. *Klin. Wchnschr.*, 36: 151, 1958.
 113. REYNOLDS, J., FOLLETTE, J. H. and VALENTINE, W. N. The arginase activity of erythrocytes and leucocytes with particular reference to pernicious anemia. *J. Lab. & Clin. Med.*, 50: 78, 1957.
 114. SCHNEIDER, R. G., LEVIN, W. C. and HAGGARD, M. E. Carbonic anhydrase activity in sickle cell anemia, sickle cell trait, and pernicious anemia. *J. Lab. & Clin. Med.*, 34: 1249, 1949.
 115. SABINE, J. C. Erythrocyte cholinesterase titers in hematologic disease. *Am. J. Med.*, 27: 81, 1959.
 116. PRITCHARD, J. A. and WEISMAN, R. Erythrocyte cholinesterase activity in normal pregnancy and in megaloblastic and other anemias of pregnancy and the puerperium. *J. Lab. & Clin. Med.*, 47: 98, 1956.
 117. SELWYN, J. G. and DACIE, J. V. Autohemolysis and other changes resulting from the incubation *in vitro* of red cells from patients with congenital hemolytic anemia. *Blood*, 9: 414, 1954.
 118. HOLLINGSWORTH, J. W. Erythrocyte glycolysis in hemolytic disease. *J. Lab. & Clin. Med.*, 45: 920, 1955.
 119. AUDITORE, J. V., HARTMANN, R. C. and COLE, E. F. Potassium transport in the acetylcholinesterase-deficient erythrocytes of paroxysmal nocturnal hemoglobinuria (PNH). *J. Clin. Invest.*, 38: 702, 1959.
 120. STORTI, E., VACCARI, F. and BALDINI, E. Changes in red cells metabolism in presence of incomplete antibodies. *Experientia*, 12: 108, 1956.
 121. BEUTLER, E. The hemolytic effect of primaquine and related compounds: a review. *Blood*, 14: 103, 1959.
 122. BAIER, H. Über die Wirkung von Blei auf die Fermentsynthese *in vivo*. *Klin. Wchnschr.*, 36: 970, 1958.
 123. VACCARI, F., TRALDI, A. and LEO, G. C. Inhibition of blood cell glycolysis in hypersplenism. *Acta haemat.*, 21: 166, 1959.
 124. REES, S., SCHEITLIN, W. G., POND, J. C., MACMANUS, T. J., GUILD, W. R. and MERILL, J. P. The effect of dialysis and purine ribosides upon the anemia of uremia. *J. Clin. Invest.*, 36: 923, 1957.
 125. ALLISON, A. C. Acute haemolytic anaemia with distortion and fragmentation of erythrocytes in children. *Brit. J. Haemat.*, 3: 1, 1957.
 126. BOCK, H. E., KARGES, O., LÖHR, G. W. and WALLER, H. D. Fermenteigenschaften roter Blutzellen bei Lebererkrankungen. *Klin. Wchnschr.*, 36: 49, 1958.

127. LÖFGREN, O. The concentration of adenine in red blood cells in cirrhosis of the liver. *Acta haemat.*, 12: 35, 1954.
128. WOLFE, S. J., BRIN, M. and DAVIDSON, C. S. The effect of thiamine deficiency on human erythrocyte metabolism. *J. Clin. Invest.*, 37: 1476, 1958.
129. GRAUBARTH, H., MACKLER, B. and GUEST, G. M. Effects of acidosis on utilization of glucose in erythrocytes and leucocytes. *Am. J. Physiol.*, 172: 301, 1953.
130. MACKLER, B. and GUEST, G. M. Blood glycolysis: mechanisms inhibited by acidosis. *Am. J. Physiol.*, 174: 269, 1953.
131. HELLEM, A. J. and SKAUG, O. E. Paroxysmal nocturnal hemoglobinuria. II. Permeability and phosphate turnover in the red blood cells. *Scandinav. J. Clin. & Lab. Invest.*, 7: 121, 1955.
132. BRANSON, H. and BANKS, L. O. The turnover time of phosphorus in normal, sickle cell trait, and sickle cell anemia blood *in vitro* as measured with P^{32} . *Science*, 115: 89, 1952.

Clinical Studies

Stimulation of the Carotid Sinus in Man*

I. The Cerebral Response

II. The Significance of Head Positioning

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THE reports of Weiss and associates [1,2] have been the foundation upon which most clinical work on carotid sinus reflex hypersensitivity has rested. These investigators distinguished three types of positive response to digital compression and massage of the carotid sinus: (1) cardioinhibition with bradycardia or asystole, (2) vasodepression unassociated with cardiac slowing, and (3) a cerebral effect characterized by facial pallor, dizziness, syncope, focal convulsions and various other temporary neurologic abnormalities independent of change in blood pressure or cardiac rate. Ferris, Capps and Weiss [2] detailed their observations on the third, or cerebral form, and concluded that the neurologic signs and symptoms which they elicited during sinus stimulation were the result of reflex vasoconstriction of cerebral blood vessels. They postulated that the carotid sinus contained afferent endings which were stimulated by pressure and massage to discharge over a reflex arc consisting of glossopharyngeal afferents and sympathetic vasoconstrictor efferents to cerebral arteries. When this work appeared Ask-Upmark [3] stressed the possibility that the results obtained by Weiss need not necessarily be reflex in nature but might, in fact, be related to mechanical interference with carotid blood flow during sinus manipulation.

More recently, Webster and Gurdjian [4], among others [5,6] have pointed out that in patients with underlying disease of the carotid arteries or of their intracranial branches, mechanical impairment of blood flow by carotid sinus compression can produce signs and symptoms identical with those postulated by Weiss to be the clinical expression of a cerebral vasoconstrictor reflex.

However, no systematic clinical investigation of the relationship, if any, between the cerebral form of carotid sinus reflex hypersensitivity postulated by Weiss, and the effects of carotid compression upon patients with the newly recognized syndromes of cerebrovascular insufficiency [7] and disease of the internal carotid artery [8] has yet been undertaken. We are attempting to do this and the current report is a summary of our findings to date.

REVIEW OF PREVIOUS WORK

In reviewing the clinical literature on carotid sinus stimulation it is immediately apparent that investigators have used different technics for elicitation of the carotid sinus reflex [9-12]. For the most part, descriptions of method used for stimulating the carotid sinus do not make clear whether the massage and pressure necessary to stimulate afferent nerve endings at the sinus did or did not compromise carotid blood flow as well. To quote Ferris, Capps and Weiss [2], "The bulbar dilatation at the bifurcation was first located. It was then manipulated until it could be held firmly over the cervical spine when *fairly strong pressure* with gentle massage was applied, using the spine as a support." One surmises from this description of technic that the tests did in fact employ pressure sufficient to impair carotid blood flow. This method is advocated by various texts of cardiology [13,14]. On the other hand, some investigators have stressed light massage without pressure [9]. Gurdjian and Webster [15] and Mackay [6] have emphasized the need for distinguishing between sinus massage and arterial compression. In order to establish a standard technic and to have

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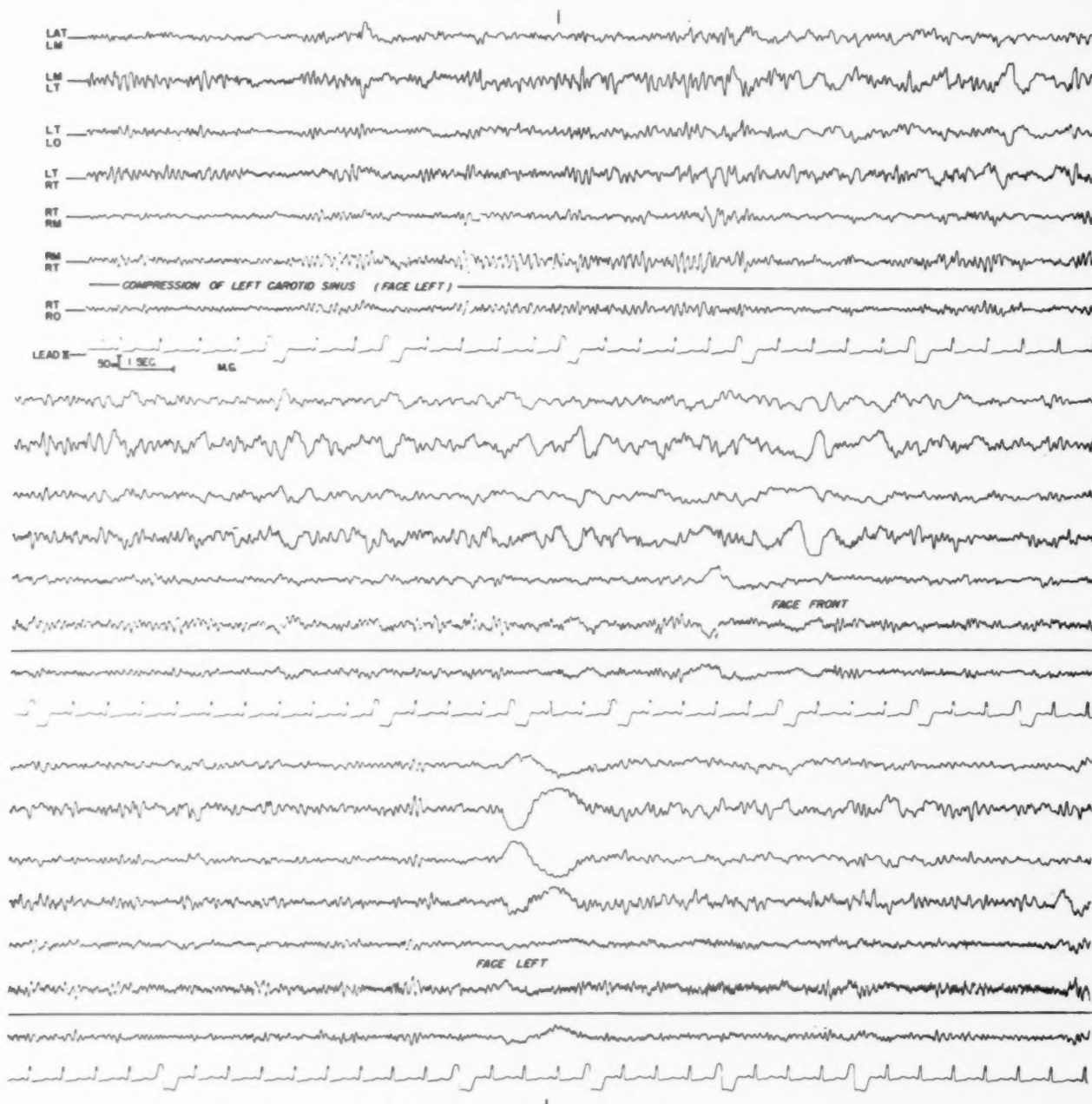


FIG. 1. Electrocardiogram illustrating the critical influence of head position upon response to continuous internal carotid sinus compression. The patient is in a horizontal position. Blood pressure 180.80 mm. Hg and stable. No abnormality occurred during preceding or subsequent carotid sinus compression with the face frontward. head II of the electrocardiogram also is recorded.

some indication of the degree of impairment of carotid blood flow during sinus testing, we palpate the ipsilateral superficial temporal artery, using presence or absence of pulsation in it as a guide to whether carotid manipulation in the neck has impaired distal blood flow. If temporal arterial pulsation continues throughout the test, we classify this as massage; if it ceases, we term this compression. While we recognize that alteration of pulsation in external carotid

branches does not necessarily signify change in flow through the internal carotid, the assumption does not seem unwarranted. In using this technic we were surprised to discover how little digital pressure was required at the sinus to obliterate palpable pulsation in the distal temporal artery.

In the past, investigators have compressed the common carotid below the sinus for control observations on sinus reflex hypersensitivity.

That is, if a response was elicited when the carotid sinus was compressed or massaged, but not when the common carotid below was similarly manipulated, it was concluded that the sinus test had stimulated afferent endings located only at the carotid sinus. However, conclusions concerning sinus reflex activity based on this logic must be reviewed because Sweet and associates [16-18] have shown that at least in some patients (e.g., patient G. D. M. [17]) carotid sinus occlusion lowers internal carotid blood pressure far more than a similar maneuver applied to the common carotid below, because collateral circulation from the external into the internal carotid can develop in the latter but not in the former instance. Therefore, cerebral effects which are obtained by compression at the internal carotid sinus, but not over the common carotid, can be due either to more complete interruption of carotid blood flow when the sinus is compressed or to a reflex initiated by sinus pressure.

Another variable which has not been considered in the past is the influence of position of the head upon the results obtained with carotid sinus compression. In describing techniques for testing for carotid sinus reflex hypersensitivity, reference is made by some workers to extending the head and turning it away from the side being tested [11]. Others do not mention head position or else advocate no turning of the head whatsoever [9,10]. However, we have been able repeatedly to obtain a response to carotid compression which varies remarkably with head position. Figure 1 illustrates this point. Note that with the face turned to the left, compression of the left carotid sinus caused theta and delta waves to appear in the electroencephalographic recording taken over the left hemisphere. This abnormality subsided almost immediately when the face was turned front and reappeared when the face was once again turned to the left. Special care was taken to maintain steady carotid pressure during position change as manifested by continued lack of pulsation in the superficial temporal artery throughout the test.

METHOD

Because the several factors mentioned have received little if any attention in previous work, we have adapted the tilt table electroencephalographic technic described by Meyer, Leiderman and Denny-Brown [19] in an attempt to assess the importance of as many variables as possible. With the patient lying horizontal on the tilt table and after baseline electro-

encephalographic recording, electrocardiogram and brachial blood pressure had been stable for about ten minutes, one internal carotid sinus was compressed posteriorly against the cervical spine for thirty seconds. If neither electroencephalographic or clinical affect was produced, we proceeded to the opposite sinus. If any abnormality was obtained with compression, gentle massage without compression (as manifest by continuation of the superficial temporal pulsation) was applied for thirty seconds. Lastly, the ipsilateral common carotid was then compressed for thirty seconds. When a cardioinhibitory or vaso-depressor response occurred, pressure was either applied more slowly (but not more gently) or atropine sufficient to block this undesired reflex was given, usually about 0.4 to 0.6 mg. intravenously. This sequence was repeated on each side with face forward, then with the face turned to the right, and finally to the left. If no abnormality was elicited with any of these maneuvers, the entire series of steps was repeated after the patient had been tilted rapidly (about ten seconds) to a head-up position 70 degrees from the horizontal position and after blood pressure and cardiac rate had again stabilized.

RESULTS

So far, we have performed these tests on thirty-nine patients, all with transitory and recurrent episodes which could be consistent with diagnoses of the cerebral form of hypersensitive carotid sinus described by Weiss, or with carotid artery disease or with so-called cerebrovascular insufficiency. We did not attempt to obtain information on "normal" people or on those suspected of having other forms of intracranial pathology.

We found only two patients who had electroencephalographic abnormalities other than minor amplitude suppression from tilting to 70 degrees. This is in accord with the findings of Weiss and Froelich [20]. Several patients said that they experienced dizziness or clouding of consciousness with turning of the head or upon looking upwards. In none of these were abnormal electroencephalographic potentials or clinical symptoms evoked by having the patient duplicate these positions during the tests. Nor did turning of the head alone produce any neurologic symptoms or the electroencephalogram change in any of our patients. In contrast, four patients had electroencephalographic changes secondary to cardioinhibition during gentle carotid massage. This response to massage never occurred when these patients were retested after vagal block with atropine.

In five of our thirty-nine patients, internal

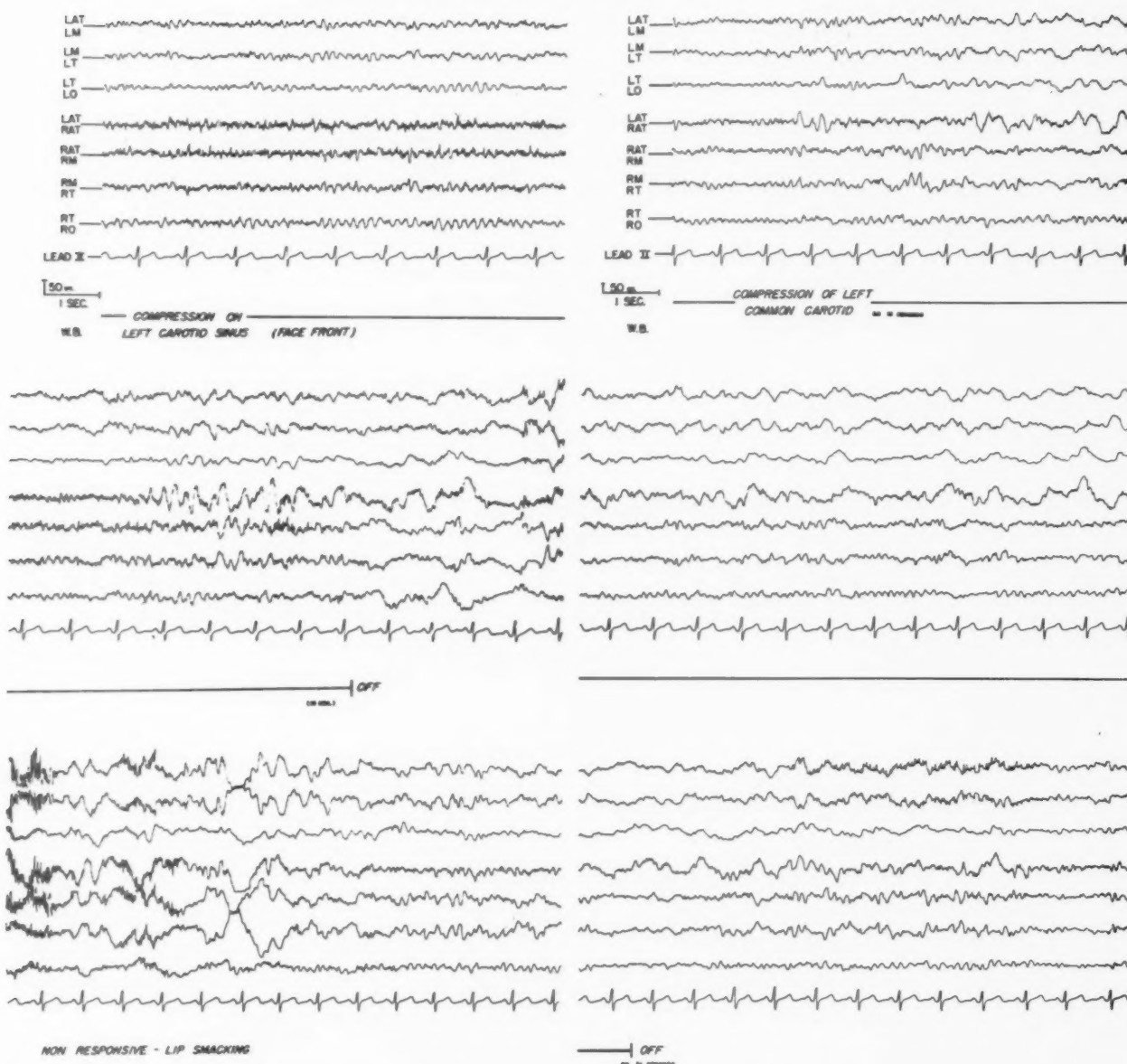


FIG. 2. To illustrate that left cervical sympathetic block and procaine infiltration of the left carotid sinus adventitia does not modify the effectiveness of left carotid sinus compression. W. B., a sixty-two year old patient, in a horizontal position. Blood pressure 220/210 mm. Hg and stable. Right-sided focal motor seizures one month before testing. Retinal artery pressures equal in both eyes. Bilateral carotid arteriography with limited dosage showed no abnormality of internal carotids. *Left*, compression of procainized carotid sinus for sixteen seconds. *Right*, compression of common carotid for thirty-one seconds. Electroencephalogram standardization, 50 microvolts equivalent to 7 mm.

carotid sinus compression while the patient was in a horizontal position with the face frontward produced facial pallor, then electroencephalographic and neurologic abnormality. Turning of the head with sinus compression evoked abnormality in five more patients who had negative responses to testing with the face frontward. When the sequence was repeated in the 70 degree position on those who had had no reaction while horizontal, only one other patient reacted with his face turned frontward; how-

ever, turning of the head added six more. Thus the total number of patients in whom a cerebral response to carotid sinus pressure developed was seventeen. In all but four of these reactors a response identical with that obtained over the internal carotid sinus was elicited by similar compression of the common carotid, i.e., the effect was not specific to the sinus. Furthermore, in two of these positive reactors procaine was infiltrated into the sinus adventitia and at the same time the ipsilateral cervical sympathetics

were blocked. The response to sinus pressure was not modified by these procedures so that a hypersensitive sinus reflex arc cannot be implicated in these patients. (Fig. 2.)

The sequence of events in all reactors was as follows: After three to twenty seconds of compression a slight increase in amplitude of basic rhythm over the symptomatic hemisphere was replaced by progressively higher voltage theta and delta waves, first over one and then over both hemispheres. At times, the dysrhythmia appeared first over the ipsilateral and in other instances on the side contralateral to the carotid sinus being tested. Concomitant with this, facial pallor almost always became manifest. Two to five seconds after definite slowing had occurred, the patient usually lost consciousness and frequently had convulsive phenomena, either focal motor or psychomotor. This sequence is similar to that described by Weiss [2]. Because of reports of permanent neurologic residua following prolonged carotid manipulation [21], we terminated compression as soon as well developed slowing of brain potentials occurred. This usually avoided a clinical seizure, and using this end point we have had no complications whatever from the test.

COMMENTS

Up to this time we have not had an example of a cerebral response to carotid sinus stimulation in which impairment of blood flow has not been evidenced by cessation of temporal artery pulsation. Furthermore, compression of the common carotid low in the neck produced a response identical with that obtained at the sinus in all but four of our seventeen patients. Procaine was infiltrated to block a postulated reflex arc in two of these four patients, but no modifying effect on the cerebral response to sinus compression occurred. These findings would seem to indicate that the response to carotid sinus manipulation which we observed in our patients was secondary to impairment of cerebral blood flow by carotid compression rather than a reflex cerebral vasoconstriction initiated by sinus stimulation. This thesis receives support from the extensive clinical studies of Gurdjian and Webster [22] and from the scant evidence for such a reflex arc in animals. Recent reviews by Aviado and Schmidt [23] and by Heymans and Neil [24] minimize the importance of reflex vasoconstriction ini-

tiated at the carotid sinus, although others [25,26] have reported some evidence to the contrary. There seems to be general agreement, however, that in animals direct cervical sympathetic stimulation can cause cerebral vasoconstriction [27]. Since thorough procaine infiltration of the sinus region and of the cervical sympathetics had no blocking effect upon the patient's response to carotid sinus compression, we do not attribute the results which we observed to reflex vasoconstriction, but rather to a mechanical interference with carotid blood flow.

Twelve of our seventeen patients with positive response to sinus compression had only one "sensitive" sinus. Arteriography or carotid surgery was performed in ten of these patients and the carotid opposite to the sensitive one was found to be occluded or severely stenosed in every one. This finding confirms the observations of Skillicorn and Aird [28] that, if one carotid is diseased, digital compression of the other, as in testing for carotid sinus hypersensitivity, can produce clinical responses identical to those ascribed to a cerebral form of carotid sinus reflex hypersensitivity in the past.

Perhaps the most important product of our investigation is the observation that position of the head during carotid sinus compression can be the critical factor in production of cerebral effects therefrom. In many patients who tolerated sinus compression with the face frontward neurologic abnormality developed when the head was turned. We have seen a variety of responses; some patients tolerate carotid compression with head positioning to one but not the other side. Tatlow and Bammer [29] have suggested that the right vertebral artery is compressed at the atlantoaxial joint when the head is turned to the left and that the opposite vessel is similarly compressed with the head turning to the right. Hutchinson and Yates [30], in an excellent clinicopathological study, have pointed out that the vertebral artery is frequently impinged upon in its canal within the cervical spine by osteophytes of osteoarthritis, so that vertebral blood flow can be impaired by extravascular disease. This work strengthens our hypothesis that the variation in response to carotid compression which we observed with testing in different head positions is dependent, at least in part, upon changes in flow through the vertebral vessels. It may be that in the classic case of the celluloid collared street car motorman, whose attacks of carotid sinus syncope occurred

when he looked to one but not the other side, the collar was not stimulating a hypersensitive carotid sinus reflex arc, but rather was impeding flow through his carotid, while at the same time head turning obstructed vertebral flow. Of importance from the clinical point of view are the reports of positional vertigo and basilar artery syndromes which seem to depend upon or to follow positioning of the head [37]. It is even conceivable that some cerebral infarctions which are first evident upon awakening might partially be the result of head position during sleep.

SUMMARY

An attempt has been made to determine the relationship between the cerebral form of carotid sinus reflex hypersensitivity described by Weiss and the more recently recognized syndrome of carotid artery insufficiency. Of thirty-nine patients with syndromes compatible with either, seventeen had a primary neurologic response to carotid sinus compression. No cerebral response was ever obtained with sinus compression insufficient to impair carotid blood flow. Ten of the twelve reactors who had only one sensitive sinus were further studied and all had marked stenosis or occlusion of the opposite internal carotid.

The previously unrecognized influence of head position on response to carotid compression tests is demonstrated. Vertebral artery compression produced by turning the head is postulated to be the basis for this result.

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REFERENCES

1. WEISS, S. and BAKER, J. P. The carotid sinus reflex in health and disease. Its role in the causation of fainting and convulsions. *Medicine*, 12: 297-354, 1933.
2. FERRIS, E. B., CAPPS, R. B. and WEISS, S. Carotid sinus syncope and its bearing on the mechanism of the unconscious state and convulsion. *Medicine*, 14: 377-456, 1935.
3. ASK-UPMARK, E. Carotid sinus and cerebral circulation. *Acta psychiat. et neurol. Scandinav.*, (supp. 6), pp. 1-374, 1935.
4. WEBSTER, J. E., GURDJIAN, E. S. and MARTIN, F. A. Mechanism of syncope due to unilateral compression of carotid bifurcation. *Arch. Neurol. & Psychiat.*, 74: 556-558, 1955.
5. OCHS, L., SENSENBACH, W. and MADISON, L. Primary thrombosis of the internal carotid artery. *Am. J. Med.*, 17: 374, 1954.
6. MACKAY, R. P. Discussion. *Tr. Am. Neurol. A.*, p. 67, 1956.
7. CORDAY, E. and ROTHENBERG, S. F. The clinical aspects of cerebral vascular insufficiency. *Ann. Int. Med.*, 47: 626, 1957.
8. FISHER, M. Occlusion of the carotid arteries: further experiences. *Arch. Neurol. & Psychiat.*, 72: 187, 1954.
9. GREELEY, H. P., SMEDAL, M. I. and MOST, W. The treatment of the carotid sinus syndrome by irradiation. *New England J. Med.*, 252: 91-94, 1955.
10. DRAPER, A. J. The cardioinhibitory carotid sinus syndrome. *Ann. Int. Med.*, 32: 700-716, 1950.
11. EVANS, E. The carotid sinus; its clinical importance. *J. A. M. A.*, 149: 45-50, 1952.
12. GAMMON, G. D. The carotid sinus reflex in patients with hypertension. *J. Clin. Invest.*, 15: 153, 1936.
13. FRIEDBERG, C. K. Diseases of the Heart, 2nd ed., p. 313. Philadelphia, 1956. W. B. Saunders Co.
14. WHITE, P. D. Heart Disease, 4th ed., p. 53. New York, 1956. Macmillan Co.
15. GURDJIAN, E. S., WEBSTER, J. E. and MARTIN, F. A. Studies of compression of the carotid bifurcation by cinematography. *Tr. Am. Neurol. A.*, pp. 66-68, 1956.
16. SWEET, W. H. and BENNETT, H. S. Changes in internal carotid pressure during carotid and jugular occlusion and their clinical significance. *J. Neurosurg.*, 5: 178-195, 1948.
17. SWEET, W. H., SARNOFF, S. J. and BAKAY, L. A clinical method for recording internal carotid blood pressure: significance of changes during carotid occlusion. *Surg., Gynec. & Obst.*, 90: 327, 1950.
18. BAKAY, L., and SWEET, W. H. Cervical and intracranial intraarterial pressures with and without vascular occlusion. *Surg., Gynec. & Obst.*, 95: 67, 1952.
19. MEYER, J. S., LEIDERMAN, H. and DENNY-BROWN, D. Electroencephalographic study of insufficiency of the basilar and carotid arteries in man. *Neurology*, 6: 455, 1956.
20. WEISS, S. and FROELICH, W. Tilt table electroencephalography in insufficiency syndromes. *Neurology*, 8: 686-693, 1958.
21. ASKEY, J. M. Hemiplegia following carotid sinus stimulation. *Am. Heart J.*, 31: 131, 1946.
22. GURDJIAN, E. J., WEBSTER, J. E. and LINDNER, D. W. On the non-existence of the cerebral form of "irritable carotid sinus." *Tr. Am. Neurol. A.*, p. 49, 1958.
23. AVIADO, D. M. and SCHMIDT, C. F. Reflexes from stretch receptors in blood vessels, heart and lungs. *Physiol. Rev.*, 35: 247, 1955.
24. HEYMANS, C. and NEIL, E. Reflexogenic Areas of the Cardiovascular System. Boston, 1958. Little, Brown & Co.
25. BONVALLET, M., DELL, P. and HIEBEL, G. Tonus sympathetique et activite electrique corticale. *Electroencephalog. Clin. Neurophysiol.*, 6: 119, 1954.

26. MAZZELLA, H., MULLIN, R. and ANSTT, E. Effect of carotid sinus stimulation on the EEG. *Acta neurol. latinoamer.*, 3: 361, 1957.
27. SCHMIDT, C. F. *The Cerebral Circulation in Health and Disease*. Springfield, Ill., 1950. Charles C Thomas.
28. SKILLICORN, S. A. and AIRD, R. B. Electroencephalographic changes resulting from carotid artery compression. *Arch. Neurol. & Psychiat.*, 71: 367-376, 1954.
29. TATLOW, W. F. and BAMMER, H. G. Syndrome of vertebral artery compression. *Neurology*, 7: 331, 1957.
30. HUTCHINSON, E. C. and YATES, P. O. The cervical portion of the vertebral artery: a clinico-pathological study. *Brain*, 80: 319, 1957.
31. SCHWARZ, G. A., GEIGER, J. K. and SPANO, A. V. Posterior inferior cerebellar artery syndrome of Wallenberg after chiropractic manipulation. *Arch. Int. Med.*, 97: 352, 1956.

Studies on the Nature of the Increased Serum Acid Phosphatase in Gaucher's Disease*

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THE presence of an elevated serum acid phosphatase in Gaucher's disease, when phenylphosphate is employed as substrate in the estimation of enzyme activity, was originally noted [1,2] by the chance observation of an elevated acid phosphatase in the serum of a woman with a lytic bone lesion presumed to be due to Gaucher's disease. An alkaline phosphatase determination had been requested and inadvertently both acid and alkaline phosphatase determinations were performed. When the results were reported the serum alkaline phosphatase was found to be normal whereas the serum acid phosphatase was found to be markedly elevated. To ascertain that this was not an error, the procedure was repeated and the finding was substantiated. Eight cases of Gaucher's disease were then investigated and all patients were found to have an elevated serum acid phosphatase, ranging from 4.8 to 11.7 Gutman units, with an average of 8.5 units. A positive pathologic diagnosis by splenectomy or sternal marrow aspiration was available in every case. The characteristic clinical manifestations of splenomegaly, hepatomegaly and/or lytic bone lesions in femurs or vertebrae were variously present.

Further studies are herein reported to characterize this acid phosphatase and to distinguish it from the enzyme responsible for the elevation of the serum acid phosphatase found in carcinoma of the prostate [3] and the acid phosphatase present in erythrocytes [4]. This separation was attempted by the differential use of substrates and inhibitors.

METHODS

The serum acid β -glycerophosphatase was determined by the technic of Bodansky [5,6] and the phenylphosphatase by the method of Gutman and Gutman [7,8] as modified by Carr [9]. Determinations

were made in duplicate and many were repeated on several occasions.

Fasting blood was obtained with appropriate caution to prevent hemolysis, and the specimens were properly refrigerated. Determinations were made on the same day the blood was drawn.

Formaldehyde 0.5 per cent, copper sulfate 0.0002 M, and L-tartrate 0.01 M were used as inhibitors of erythrocytic and prostatic acid phosphatase, as described by Abul-F'Adl and King [10,11].

A Beckman glass electrode pH meter was used in pH-activity studies. At varying pH levels the activity of the elevated serum acid phosphatase in Gaucher's disease was found to be maximum at pH 5 with disodium phenylphosphate or β -glycerophosphate as substrate. In this respect, therefore, the acid phosphatases of normal erythrocyte, prostatic carcinoma and Gaucher's disease were identical, and buffers at pH 5 therefore were used throughout these studies.

For study of erythrocyte acid phosphatase activity, oxalated human red blood cells were washed several times with isotonic saline solution, then hemolyzed with distilled water, centrifuged, and the supernatant fluid was analyzed for hemoglobin to correlate concentration of red blood cells with erythrocyte enzyme activity.

RESULTS

In twelve cases of proved Gaucher's disease (Table 1) there was a range of 7 to 14.3 acid phenylphosphatase units, with an average of 9.4 units. This series included three of the previously reported cases. These values for acid phenylphosphatase, while not in the markedly elevated range frequently found in patients with carcinoma of the prostate with metastases, are all clearly elevated, as Figure 1 shows. The normal range in this laboratory does not exceed 4 to 5 Gutman units.

The values for acid β -glycerophosphatase ranged from 0.3 to 1.1 Bodansky units, with an average of 0.7 units, many of these results falling within normal limits. No correlation could be made between the results with β -glycero-

* From the Departments of Medicine and Chemistry, The Mount Sinai Hospital, New York, New York. Aided by a grant from the Damon Runyon Memorial Fund.

TABLE 1
SERUM ACID PHOSPHATASE IN GAUCHER'S DISEASE

| Patient | Sex and Age (yr.) | Bodansky Units | Gutman Units | Inhibitors Used | | | Diagnosis Confirmed by |
|---------|-------------------|----------------|--------------|-------------------|----------------------------|-------------------|---------------------------|
| | | | | Formaldehyde 0.5% | CuSO ₄ 0.0002 M | L-Tartrate 0.01 M | |
| M. L. | M, 7 | 0.75 | 10.2 | 7.9 | 10.8 | 9.9 | Sternal marrow aspiration |
| R. L. | F, 30 | 0.57 | 9.3 | 7.2 | 8.4 | 8.6 | Splenectomy |
| D. K. | F, 19 | 0.65 | 8.8 | 5.0 | 6.6 | 6.7 | Splenectomy |
| A. M. | M, 35 | 1.14 | 9.3 | 5.6 | 7.2 | 7.6 | Sternal marrow aspiration |
| L. R. | M, 45 | 1.02 | 9.7 | 6.4 | 7.8 | 8.9 | Sternal marrow aspiration |
| H. S. | M, 10 | 0.50 | 9.6 | 4.2 | 7.4 | 7.6 | Sternal marrow aspiration |
| A. A. | F, 27 | 0.88 | 14.3 | 11.4 | 13.0 | 13.6 | Splenectomy |
| J. D. | F, 52 | 0.29 | 8.6 | 7.1 | 8.3 | 8.0 | Splenectomy |
| B. S. | F, 47 | 0.54 | 8.2 | 5.5 | 6.6 | 7.0 | Sternal marrow aspiration |
| P. K. | F, 49 | 0.68 | 8.8 | 6.7 | 8.8 | 8.4 | Splenectomy |
| G. K. | M, 68 | 0.34 | 7.0 | 6.2 | 7.5 | 6.7 | Sternal marrow aspiration |
| L. L. | F, 52 | 0.80 | 9.5 | 10.0 | 10.0 | 7.2 | Splenectomy |

phosphate as substrate (Bodansky) and those with disodium phenylphosphate (Gutman) as substrate. For example, in Case II (Table I) values were 0.6 Bodansky units and 9.3 Gutman units, while in Case IV values were 9.3 Gutman units and 1.1 Bodansky units.

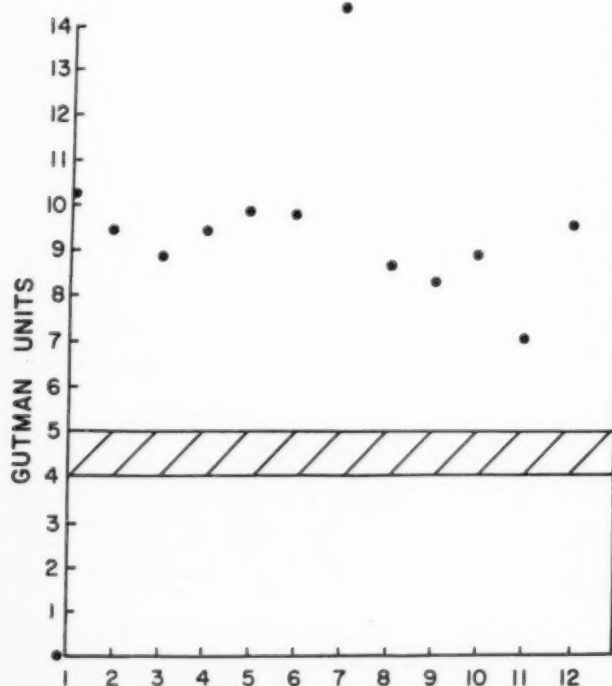


FIG. 1. Serum acid phosphatase in twelve cases of Gaucher's disease, using disodium phenylphosphate as substrate. (Normal range up to 4 units, 4 to 5 units equivocal range, over 5 units pathologic range.) Each dot represents one case.

Figure 2 shows the distribution of the serum acid phosphatase levels in the twelve cases of Gaucher's disease, using β -glycerophosphate as the substrate. The dephosphorylation of this substrate by the acid phosphatase of Gaucher's disease is slower than that of disodium phenylphosphate. This relative ease of splitting of disodium phenylphosphate as compared with β -glycerophosphate was reported by Hastrup and Videbaek [12] who found an elevated serum acid phosphatase in Niemann-Pick's disease with disodium phenylphosphate and a normal value with β -glycerophosphate as substrates.

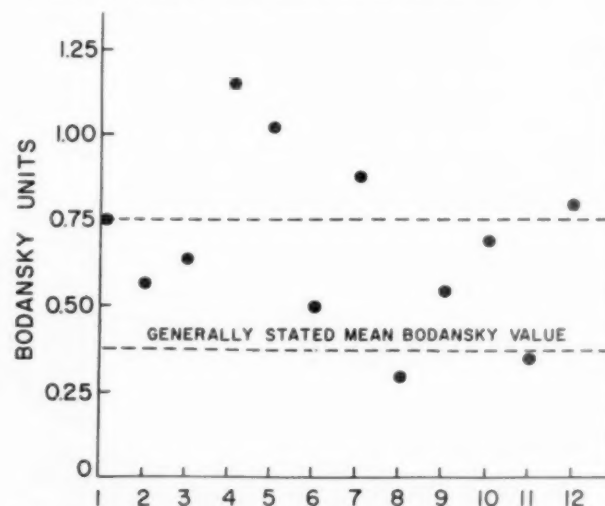


FIG. 2. Serum acid phosphatase in twelve cases of Gaucher's disease, using β -glycerophosphate as substrate. (Values over 0.75 in pathologic range.) Each dot represents one case.

TABLE II
EFFECT OF INHIBITORS ON ERYTHROCYTE ACID PHOSPHATASE

| Hemo- globin Concen- tration (gm. %) | Bodansky Units | Gutman Units | | | |
|--|-------------------|-----------------------|------|------------------|---------------|
| | | No Inhibi- tors | HCHO | Cu ⁺⁺ | Tar- trate |
| 7.1 | 1.58 | 90.4 | 3.9 | 32.6 | 90.4 |
| 8.9 | 3.7 | 126.4 | 0.3 | 68.4 | 125.6 |

TABLE III
EFFECT OF INHIBITORS ON SERUM ACID PHOSPHATASE
IN CARCINOMA OF THE PROSTATE

| Bodansky Units | Gutman Units | | | |
|-------------------|------------------|------|------------------|----------|
| | No Inhibitors | HCHO | Cu ⁺⁺ | Tartrate |
| 19.9 | 59 | 56 | 57.2 | 5.3 |
| 17.6 | 42.2 | 43.2 | 43.6 | 9.0 |
| 14.8 | 35.2 | 29.4 | 34 | 5.6 |

Abul-F'Adl and King [10] have demonstrated complete or virtually complete inhibition of the acid phenylphosphatase of erythrocytes by formaldehyde (100 per cent) and to a lesser degree by copper (90 per cent). Our studies on the inhibition of erythrocytic acid phosphatase, using hemolyzed human red blood cells, confirm their findings. (Table II.) They also showed that prostatic acid phosphatase is not inhibited by these agents but is inhibited by L-tartrate. (Table III.) The serum acid phosphatase in Gaucher's disease, as shown in Figure 3, differs both from erythrocytic and prostatic acid phosphatase in that it is not significantly inhibited by L-tartrate or by copper ion, but is inhibited to a greater degree by 0.5 per cent formaldehyde.

COMMENTS

Among the past one thousand determinations of serum acid phosphatase performed in the routine clinical chemical laboratory of this Hospital by the phenylphosphatase method employed in this study, a significant increase was observed in thirty-two cases. Two of these proved to be Gaucher's disease and thirty

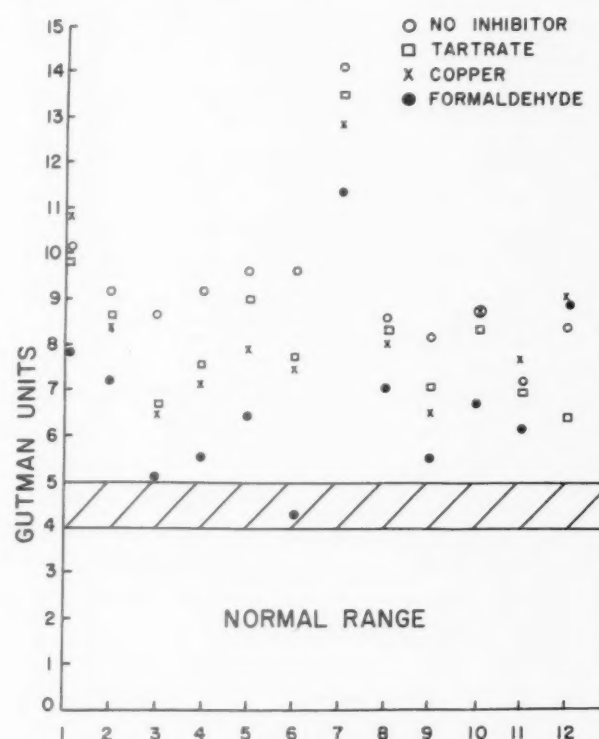


FIG. 3. Serum acid phosphatase activity in twelve cases of Gaucher's disease, using disodium phenylphosphate as substrate, with and without inhibitors.

carcinoma of the prostate. Of course it must be remembered that almost invariably the clinical suspicion or condition to be excluded was carcinoma of the prostate. If the test were ordered over a wider spectrum of diseases, it is possible that elevations from causes other than Gaucher's disease would have appeared. The occasional finding of an unexplained elevation has long been known. For example, we have followed a sixty year old man with gout for well over a year and have never explained repeated values ranging from 12 to 18 Gutman units. This patient has not shown any evidence of Gaucher's disease, and sternal marrow aspiration was negative. Many other patients with gout were found to have normal values.

Abul-F'Adl and King demonstrated that the tissue acid phosphatase of prostate, liver and spleen is strongly inhibited by L-tartrate. If the elevated serum acid phosphatase in carcinoma of the prostate is derived from the prostate gland, as has been well established, one might infer that the elevation of the serum acid phosphatase in Gaucher's disease is derived from spleen, liver or bone marrow, which are primarily involved in this disease. However, these organs do not appear to be the source of the

increased serum acid phosphatase in Gaucher's disease, since our data indicate that the circulating enzyme is not inhibited by L-tartrate.

An elevated serum acid phosphatase which is not markedly inhibited by L-tartrate, formaldehyde or copper should raise the clinical suspicion of Gaucher's disease, and if splenomegaly or hepatomegaly are present sternal marrow aspiration should be performed.

CONCLUSIONS

1. The serum acid phosphatase is increased in Gaucher's disease, when disodium phenylphosphate is used as substrate.
2. The acid phosphatase of Gaucher's disease is not inhibited by L-tartrate or copper, and is only slightly inhibited by formaldehyde, thus characterizing it as different from erythrocyte acid phosphatase and the phosphatases originating in liver, prostate, spleen or bone marrow.
3. The finding of an elevated serum acid phosphatase which is not inhibited by L-tartrate, formaldehyde or copper should suggest the possibility of inapparent Gaucher's disease, or serve to support the diagnosis in suspected cases.

REFERENCES

1. TUCHMAN, L. R., SUNA, H. and CARR, J. J. Elevation of serum acid phosphatase in Gaucher's disease. *J. Mt. Sinai Hosp.*, 23: 227, 1956.
2. TUCHMAN, L. R. and SWICK, M. High acid phosphatase level indicating Gaucher's disease in a patient with prostatism. *J. A. M. A.*, 164: 2034, 1957.
3. GUTMAN, A. B. and GUTMAN, E. B. An acid phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostate gland. *J. Clin. Invest.*, 17: 473, 1938.
4. GUTMAN, A. B. and GUTMAN, E. B. Erythrocyte phosphatase activity in hemolyzed sera and estimation of serum acid phosphatase. *Proc. Soc. Exper. Biol. & Med.*, 47: 513, 1941.
5. BODANSKY, A. Phosphatase studies. II. Determination of serum phosphatase. Factors influencing the accuracy of the determination. *J. Biol. Chem.*, 101: 93, 1933.
6. SHINOWARA, G. Y., JONES, L. M. and REINHART, H. L. The estimation of serum inorganic phosphate and "acid" and "alkaline" phosphatase activity. *J. Biol. Chem.*, 142: 921, 1942.
7. GUTMAN, A. B. and GUTMAN, E. B. Acid phosphatase activity of the serum of normal human subjects. *Proc. Soc. Exper. Biol. & Med.*, 38: 470, 1938.
8. GUTMAN, E. B. and GUTMAN, A. B. Estimation of acid phosphatase activity of blood serum. *J. Biol. Chem.*, 136: 201, 1940.
9. CARR, J. J. Alkaline and Acid Phosphatase. Standard Methods of Clinical Chemistry, vol. 1, p. 75. New York, 1953. Academic Press.
10. ABUL-F'ADL, M. A. M. and KING, E. J. The inhibition of acid phosphatases by formaldehyde and its clinical application for the determination of serum acid phosphatases. *J. Clin. Path.*, 1: 80, 1948.
11. ABUL-F'ADL, M. A. M. and KING, E. J. Properties of the acid phosphatases of the erythrocytes and the human prostate gland. *Biochem. J.*, 45: 51, 1949.
12. HASTRUP, B. and VIDEBAEK, A. "Acid" phosphatase in Niemann-Pick's disease and a therapeutic experiment with cortisone. *Acta med. Scandinav.*, 149: 287, 1954.

Hypoproteinemia Antedating Intestinal Lesions, and Possibly Due to Excessive Serum Protein Loss into the Intestine*

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HYPOPROTEINEMIA may result from a variety of causes. These include starvation, inadequate digestion or intestinal absorption which may accompany pancreatic insufficiency and disorders of the intestine, the insufficient protein synthesis of liver diseases and agammaglobulinemia, and loss of protein due to hemorrhage, burns or renal disease. There are hypoproteinemias apparently of congenital origin [1,2], and a transient hypoproteinemia of infants [3]. One group of patients has diminished serum protein levels which are unexplained. "Idiopathic hypoproteinemia" has been the classification applied most commonly to these persons.

Albright and his associates were the first to present evidence that hypoproteinemia could be associated with rapid disappearance of proteins from plasma [4]. After a series of ingenious experiments on a patient with unexplained hypoproteinemia, they concluded that excessive catabolism of serum protein was responsible for the low protein levels. Schwartz subsequently reported two cases of hypoproteinemia in which albumin, labelled with I^{131} , disappeared from the plasma at an accelerated rate [5].

Loss of serum proteins into the intestinal lumen in patients with regional enteritis and ulcerative colitis has recently been demonstrated by Steinfield et al. [6]. Their patients were found to have an accelerated disappearance of radioactive albumin from plasma, increased radioactivity in the stools when diarrhea was present, and identifiable albumin and gamma globulin in the intestinal fluids. Citrin et al. studied a case of hypoalbuminemia with hypertrophic gastritis and also found rapid disappearance of radio-

iodinated albumin from the plasma as well as radioactive albumin in the gastric juice [7].

Gordon has synthesized a radioiodinated polyvinylpyrrolidone (PVP) with a molecular weight approximating that of albumin and which is not digested by intestinal enzymes [8]. When this material was administered intravenously to patients with various intestinal diseases or with idiopathic hypoproteinemia, a larger amount appeared in the stools than is recovered from control subjects. The increase in PVP excretion was roughly proportional to the severity of the hypoproteinemia. It has therefore been inferred that the hypoproteinemia may be a consequence of an unusual loss of serum protein into the intestine [8-10].

The purpose of this paper is to report studies conducted in recent years on six patients with idiopathic hypoproteinemia, in three of whom distinct intestinal lesions have developed. In all six patients serum albumin and γ -globulin levels were depressed, and labelled samples of both proteins disappeared from the plasma at an abnormally rapid rate. Albumin, γ -globulin and other serum proteins have been found in the intestinal secretions of some of these patients and of normal persons as well. The results suggest that serum proteins normally enter the intestinal fluid. They are also compatible with the view that hypoproteinemia may be a consequence of excessive loss of serum protein into the intestine.

CASE REPORTS

(The clinical data are summarized in Table I and the laboratory data in Table II.)

CASE I. In 1936, when the patient (B. G.) was twenty-five years of age, peripheral edema and inter-

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TABLE I
CLINICAL DATA ON PATIENTS

| Case No., Year of Birth and Sex | Year of Disease Onset | Duration of Dis- ease before Localized Gastrointestinal Lesion Appeared (yr.) | Edema | Diarrhea | Vomit- ing | Pain | Fever | Weight Loss | Abnormal Small Bowel Seen on X-ray Film | Abnormal Results of Barium Enema | Melena | Steat- orrhoea | Dimin- ished B-12 Absorp- tion | Dimin- ished Glucose Absorp- tion | Abnormal Results of Jejunal Biopsy | Lesion Found at Lapa- rotomy | Susceptibility to Infection |
|--|--------------------------------|--|-------|----------|---------------|------|-------|----------------|--|---|--------|-------------------|--|---|---|--|--------------------------------|
| I, 1911, F | 1936 | 17 | + | + | 0 | 0 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | .. | + | 0 |
| II, 1940, M | 1954 | 3 | + | + | 0 | 0 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | .. | + | 0 |
| III, 1909, F | 1939 | .. | + | + | Rare | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | .. | .. | 0 |
| IV, 1936, F | 1953 | .. | + | + | 0 | Rare | 0 | 0 | + | 0 | 0 | ± | 0 | 0 | 0 | .. | 0 |
| V, 1930, F | 1954 | .. | + | + | + | 0 | 0 | 0 | + | 0 | 0 | + | + | ± | 0 | 0 | 0 |
| VI, 1951, M | 1952 | 4 | 0 | ± | 0 | + | + | + | 0 | + | + | 0 | .. | .. | .. | + | + |

* Appeared only after seventeen years, when granuloma was present.

† Present only after distinct lesion appeared.

TABLE II
LABORATORY DATA ON PATIENTS

| Case No. | Hgb (gm. %) | Red Blood Cells (million/cu. mm.) | White Blood Count and Differential | Bone Marrow | Serum | | | | | Prothrombin | Fibrinogen (mg. %) | Serum Iron (mg. %) | Iron-Binding Capacity (mg. %) | Serum Copper (mg. %) | Ceruleoplasmin | Bromsulphalein Retention | Urine Protein | Tuberculin | Kveim Test | Total Serum Cholesterol (mg. %) |
|----------|-------------|-----------------------------------|------------------------------------|----------------|-----------------------|--------------------------------|----------------|---------------|----------------|-------------|--------------------|--------------------|-------------------------------|----------------------|----------------|--------------------------|---------------|------------|------------|---------------------------------|
| | | | | | Total Protein (gm. %) | Albumin (lowest value) (gm. %) | Gamma Globulin | Beta Globulin | Alpha Globulin | | | | | | | | | | | |
| I | 11 | 4 | 6,400 N* | hyper† | 4.0 | 2.5 | low | N | N | 300 | 70 | 370 | 95 | 2.7 | 1.5 to 4.0 | 5 % | N | N | N | 150 to 240 |
| II | 11 | 4 | 7,000 N | hyper | 3.5 | 2.0 | low | N | N | 387 | 70 | 370 | 113 | 3.5 | 2.7 | N | N | N | 161 | |
| III | 11 | 3.8 | 4,000 N | hyper | 3.0 | 2.0 | low | N | N | 259 | 138 | ... | 99 | 2.6 | 3.5 | N | N | N | ... | |
| IV | 11 | 4.5 | 6,100 N | N | 3.5 | 2.4 | low | N | N | ... | 116 | 416 | 116 | 3.2 | 2.6 | N | N | N | 211 | |
| V | 10 | 4.5 | 5,000 N | N | 2.9 | 1.5 | low | low | low | ... | 60 | 366 | 70 | 1.1 | 3.2 | N | N | N | 168 | |
| VI | 10 | 3.9 | 7,000 to 27,000 Lymphocytosis | N eosinophilia | 6.5 | 1.3 | low | N | high | 325 | 84 | ... | 302 | ... | 1.1 | 12 % | N | N | N | 162 |
| | | | | | | | | | | | | | | | ... | | | | | 182 |

NOTE: Bold face figures represent normal values.

* N = normal or negative.

† hyper = minimal erythroid hyperplasia.

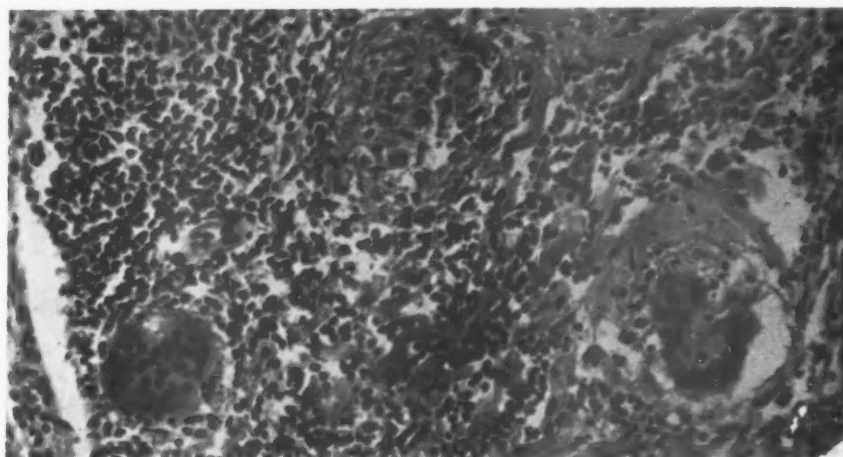


FIG. 1. Case 1. Sections of pathologic tissue. Mesenteric lymph node showing fibrosis and giant cells.

mittent diarrhea appeared. An ovarian cyst was discovered and excised. At operation the intestine appeared normal, but an enlarged mesenteric lymph node was removed which contained a non-caseating granuloma. Postoperatively, symptoms continued for fifteen years. No evidence for a tuberculous infection was found. Repeated study showed hypoproteinemia, edema, hypochromic anemia, minimal thickening of the small bowel mucosa, and normal absorption of sugar, fat and protein. The diagnosis was non-tropical sprue and she was treated with diuretics and infusions of albumin.

At the Hospital of the Rockefeller Institute in 1951 the patient had edema without diarrhea. The serum total protein was 4.3 gm. per cent with an albumin of 2.4 gm. per cent. Four years later, when again seen, a mass in the left upper quadrant was present. The serum albumin and γ -globulin were low, and labelled samples of both proteins disappeared from plasma more rapidly than normal. On abdominal exploration, only the jejunum was abnormal. Dilated and stenotic loops of bowel were present; the latter were associated with areas of constriction of the mesentery. Mesenteric nodes were cystic and the lymphatics were strikingly dilated. The mesentery was so greatly thickened and friable that excision of diseased intestine was considered inadvisable.

Biopsy specimens of small bowel, liver and adipose tissue were normal. Mesenteric nodes contained reticuloendothelial hyperplasia, fibrosis, and giant cells containing crystals. (Fig. 1.) The pathologic diagnosis was chronic non-caseating granuloma of the lymph nodes resembling Boeck's sarcoid.

Following surgery, no other evidence of sarcoid was found. Results of the Kveim test were negative. Since operation the patient has been maintained on 10 mg. of prednisone therapy daily. Despite continued hypoproteinemia and accelerated loss of plasma albumin, the patient is free of edema and ascites. As judged by its appearance on roentgenogram, the small bowel

lesion diminished initially with steroid therapy and has not changed for two years. However, there is evidence of spread of the granuloma to pelvic nodes.

CASE II. In 1954 when the patient (R. D.) was fourteen years of age, edema and diarrhea appeared. In 1955 a diagnosis of non-tropical sprue was made because of hypoalbuminemia, hypocalcemia, and an abnormal small bowel pattern with flaking of barium and loss of normal mucosal pattern.

In 1955, the patient was admitted to the Hospital of the Rockefeller Institute, with a serum albumin of 2.9 gm. per cent and reduced γ -globulin. Steatorrhea was present only with ingestion of high fat diets. Ingestion of gluten did not increase diarrhea. Albumin and γ -globulin disappeared from the plasma more rapidly than normal.

A fat-free diet reduced the diarrhea and edema considerably, and the serum albumin rose to about 4.0 gm. per cent. However, in 1957 fever, pain in the back and an abdominal mass appeared. Extension of the small bowel lesion was apparent, with dilatation and fixation of the proximal jejunum. Despite treatment with prednisone, the symptoms continued. The serum γ -globulin became elevated. Abdominal exploration revealed fixation of a portion of the proximal jejunum in a retroperitoneal and mesenteric mass. Lymphatics over the entire peritoneum were dilated. Biopsies of lymph nodes, omentum, mesentery and the mass showed only greatly dilated lymphatics and acute and chronic inflammation. (Fig. 2.) No giant cells were seen. Biopsy of the jejunal wall showed only mucosal edema. No resection was attempted. Postoperatively, the patient continued to have intermittent fever, nausea and pain in the back. Treatment with a low-fat diet, prednisone, γ -globulin, antibiotics and radiation did not improve the symptoms or the small bowel pattern.

In 1958 perforation of the affected jejunum occurred. At surgery, the appearance of the abdominal

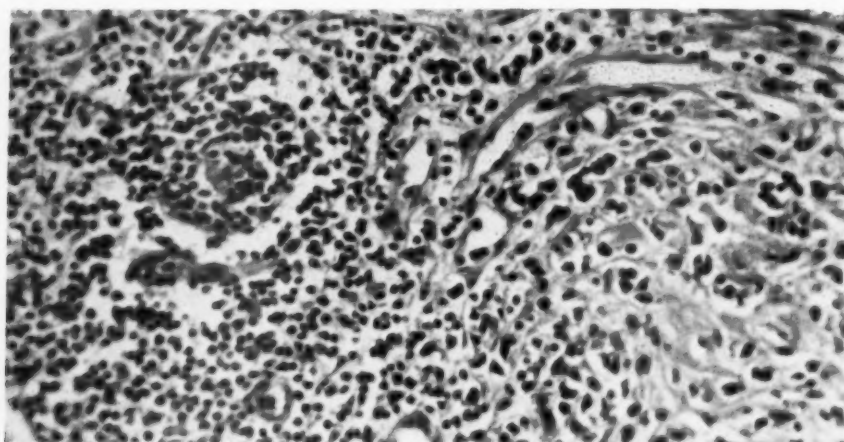


FIG. 2. Case II. Sections of pathologic tissue. Mesentery showing inflammatory cell infiltration and fibrosis.

organs and dilated lymphatics was unchanged but the spleen was enlarged. Resection of the diseased area was accomplished; some inflamed mesentery and omentum was not excised. The resected bowel and mesentery showed only edema and chronic inflammation. Following resection, all symptoms disappeared. The patient has returned to a normal diet and the serum albumin and γ -globulin have returned to normal levels.

CASE III. In 1939, edema and diarrhea appeared in patient G. P., thirty years of age. Intermittent ascites began five years later. Hypoproteinemia was found but intestinal studies were always normal. Treatment with dietary restriction, salt restriction, corticosteroids, thyroid hormone, testosterone and ACTH was not beneficial. Transfusions and albumin infusions were used until the patient became sensitive to a contaminant in commercial albumin preparations.

At age forty-six, in 1956, the patient entered the Hospital of the Rockefeller Institute, with edema and diarrhea. Hypoproteinemia was present and albumin and γ -globulin disappeared from the plasma at an accelerated rate. Small bowel studies were normal. During evaluation of the patient, she visited her home, fell, and a permanent hemiplegia and hemisensory syndrome developed. She died elsewhere and no post-mortem examination was performed.

CASE IV. In 1953, edema and occasional diarrhea appeared in patient M. C., seventeen years of age. Stools were somewhat foul. For the ensuing five years the patient was given occasional diuretics and advised to restrict salt intake and eat protein. Except for nausea, the symptoms remained unchanged and were not incapacitating. In 1958, she was seen in the Hospital of the Rockefeller Institute. Hypoproteinemia was present and a small bowel series showed dilatation of the jejunum and ileum, mucosal thickening and blunting of the valvulae conniventes. Jejunal biopsy

was normal. Albumin and γ -globulin "turnover" rates were abnormally high.

On a low fat diet, edema has been minimal and diarrhea infrequent. The patient has delivered a normal child during this illness.

CASE V. In 1954, diarrhea and peripheral edema appeared in patient N. W., twenty-four years of age. Hypoproteinemia and a coarse, segmented, small bowel mucosal pattern were found. Treatment with dietary manipulation, antispasmodics and corticosteroids did not help. Intravenous administration of albumin gave temporary relief. The diarrhea became more severe and vomiting began. The patient was studied carefully over the ensuing few years during which time rapid disappearance of albumin from the plasma was demonstrated. No abnormality was found at laparotomy (biopsy of the small bowel was not performed).

The patient entered the Hospital of the Rockefeller Institute in 1958, with vomiting, diarrhea, mild edema and hypoproteinemia. A small bowel study revealed dilatation, blunting of the valvulae conniventes, and fragmentation of the barium. Both albumin and γ -globulin disappeared from the plasma at an abnormally rapid rate. Jejunal mucosa biopsy at three sites was negative. The patient has been treated with γ -globulin, corticosteroids, estrogens, thyroid hormone, and low starch and low fat diets without benefit. Symptoms continue unabated.

CASE VI. In 1952, patient K. D., ten months of age, began to have frequent infections. In 1955 a diagnosis of cyclic neutropenia was made and splenectomy performed. However, recurrent infections continued despite immense doses of γ -globulin. A refractory gangrene appeared at a cut-down site and required a mid-thigh amputation. Examination of the bone marrow showed marked eosinophilia.

In 1956 the patient entered the Hospital of the

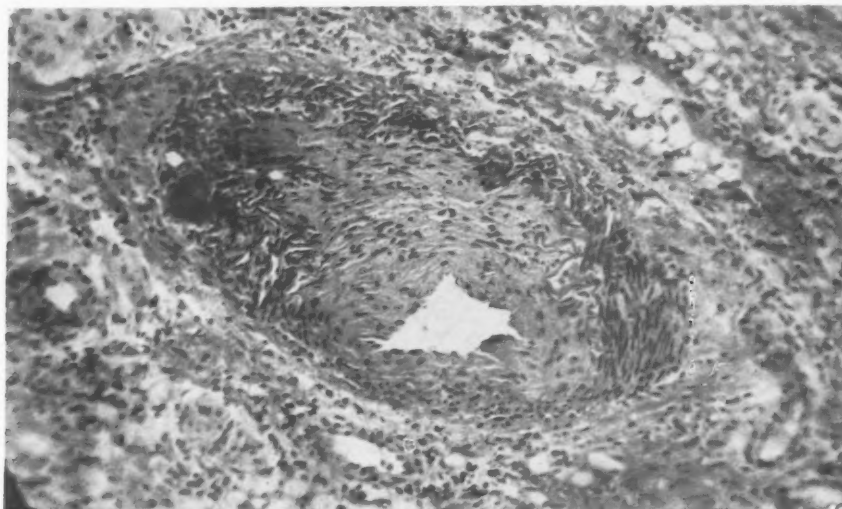


FIG. 3. Case vi. Section through an ulcer at the base of the ileocecal valve showing hypertrophy of the intima of an arteriole.

Rockefeller Institute with fever, ulceration of the buccal mucosa, and severe emaciation. He had neither diarrhea nor edema. The serum albumin and γ -globulin were depressed, and the former and probably the latter disappeared from plasma at an accelerated rate. The patient was treated symptomatically and the ulcers responded to Mycostatin.[®] A few months later melena appeared and a cecal polyp was identified. At operation there were multiple ulcers in the terminal ileum and ascending colon, with an infiltrative lesion of the gut wall extending into the mesentery. The regional nodes were enlarged. A similar lesion was present at the mid-portion of the descending colon. An internal ileotransverse colostomy was performed, with resection of diseased bowel except for that in the descending colon.

Histological examination showed extensive mucosal ulceration with acute and chronic inflammatory reaction. Arteries in the area showed extensive proliferation of the intima and leukocytic infiltration. (Fig. 3.) No specific diagnosis could be made.

Postoperatively, the patient did well. He gained weight and the serum albumin returned to normal but the γ -globulin diminished further. Steroids were given for a brief period in the hope of healing the remaining lesion in the descending colon. This did not occur, but no symptoms have appeared. In the past two years the patient has resumed his normal development. However, γ -globulin must be given at regular intervals to prevent infections.

MATERIALS AND METHODS

Labelled Serum Proteins. Human serum albumin containing approximately 1 atom of I^{131} per molecule was used.* The label was firmly bound to protein; less than 2 per cent of the radioactivity could be removed by dialysis for twenty-four hours or resisted precipita-

tion by trichloroacetic acid (TCA). The labelled albumin migrated as albumin on starch zone electrophoresis and radioactivity was present only in the albumin fraction of the subjects' serum proteins after injection of the labelled material.

Radioiodinated human γ -globulin* was also employed. However, this substance had as much as 10 per cent unbound iodine and often developed visible precipitates which presumably were denatured protein. On electrophoresis, a portion of the protein migrated as a beta globulin, and radioactivity was present in both gamma and beta globulins of the subject's serum proteins after injection of I^{131} γ -globulin.

Injection of Protein and Collection of Samples. The subjects were given fifteen drops of Lugol's solution daily for two or three days prior to injection of labelled protein in order to saturate the thyroid gland with iodine. Then a known amount of radioactivity, between 20 and 40 microcuries, was injected intravenously. Samples of heparinized blood, 10 ml., were obtained daily or on alternate days. Twenty-four-hour urine and stool collections were made.

Determination of Radioactivity and Calculations. Radioactivity was determined in a well-type scintillation counter, using 4 ml. samples of plasma, urine, intestinal fluid or homogenates of stool. The counts per minute per sample were corrected to zero time, thus eliminating the effect of decay of the label and allowing use of the data to demonstrate the biologic half-life of the protein. Counts per minute per 4 ml. plasma were plotted on a logarithmic scale against the time in days on an arithmetic scale. This semilogarithmic plot gave the typical exponential disappearance curve of radioactivity from plasma. Total injected counts were determined from appropriate standard solutions and the per cent of the total excreted daily in urine and stool could be readily estimated.

Calculations of the degradation or turnover rate of proteins, of total body albumin pool, and of the daily

* Abbott Laboratories, Chicago, Illinois.

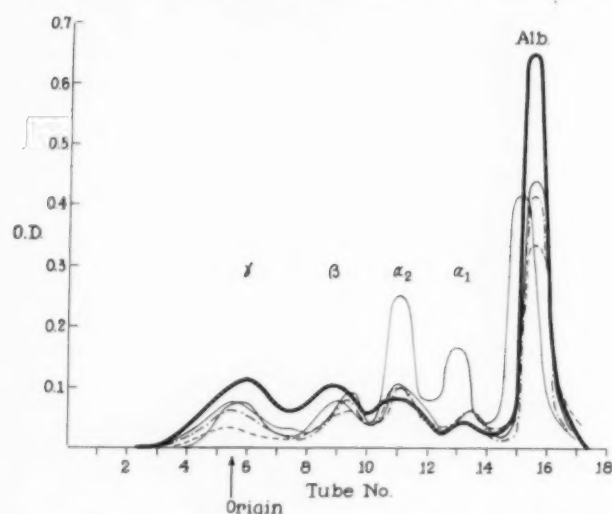


FIG. 4. Serum electrophoresis patterns of patients with hypoproteinemia (dotted and thin lines) compared with that of a normal subject (heavy line).

rate of synthesis were made by the methods outlined by Sterling [11].

Intestinal Intubation and Intestinal Fluid Sample Collection. A polyethylene intestinal tube of the type described by Hirsch et al. [12] was passed through the nasopharynx, and could be left in place for many days with minimal discomfort to the patient. A 6-inch tip was employed which was made from radiopaque materials with a greater bore than the polyethylene tube and which contained many perforations. This facilitated collection of viscous secretions and also allowed ready visualization of the position of the tube. All collections were made in the jejunum within 2 to 3 feet of the ligament of Treitz.

Intestinal fluid was collected in one of three ways: (1) into the plastic counting tube, or glass test tubes at room temperature, (2) into warm 12.5 per cent TCA, or (3) into a vessel immersed in dry ice-alcohol mixtures at -60°C . Ratios of volume of intestinal fluid to volume of TCA solution of 3:1, 2:1, and 1:1 were used. TCA precipitates were separated by centrifugation, washed twice with TCA solution, and dissolved in 1N NaOH for counting. All samples of intestinal fluid were tested for guaiac reaction. Occasionally, samples from Case IV were weakly positive. Fluid collected on that day was then discarded.

Immunological Studies. Deep frozen intestinal fluid was used for immunologic studies. The fluid was thawed at 4°C . with constant mixing in the presence of 4 to 10 mg. of soybean trypsin inhibitor per 3 to 4 ml. of intestinal fluid. The thawed fluid was immediately spun at 56,000 g for thirty minutes to remove solids and some mucus. The supernates were employed for immunologic studies. Capillary tube reactions were conducted at room temperature with the intestinal fluid placed above the antiserum. Precipitates at the interface were estimated. Agar

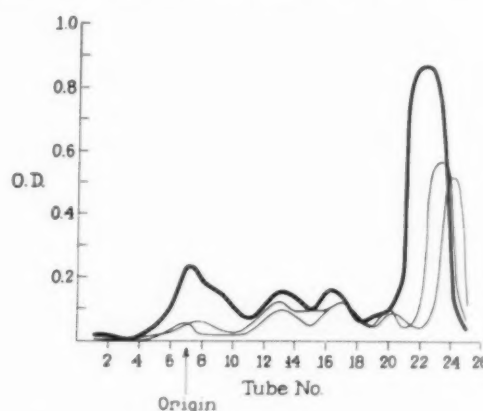


FIG. 5. Serum electrophoresis patterns of patients with hypoproteinemia (thin lines) compared with that of a normal subject (heavy line).

diffusion studies were conducted by the method of Ouchterlony, using 0.5 per cent agar [13]. All materials were kept at 4°C . throughout.

Antisera were obtained by injection of rabbits with normal whole human serum, electrophoretically separated normal human serum albumin, and normal human 7S γ -globulin (Lederle Fraction II). Some horse antiserum to whole human serum (obtained from the Pasteur Institute, Paris, France), was employed. Red Cross human albumin and Lederle Fraction II were used in the diffusion studies.

Electrophoresis. Electrophoresis was conducted on starch in barbital buffer, pH 8.6, ionic strength 0.1. Half centimeter sections were eluted in isotonic NaCl and protein concentration determined by a modification of the method of Folin-Ciocalteu [14].

Jejunal Biopsy. Suction biopsy of the proximal jejunum was performed with a Shiner tube [15] in patients 4 and 5, and was normal in each case.

RESULTS

Serum Protein Levels. Table II presents the levels of many different proteins in the patient's serums. In all patients, hemoglobin, albumin and γ -globulin were depressed. Other measured proteins had normal or low normal values except for depressed values in patient 5, who had persistent diarrhea.

Figures 4 and 5 compare the electrophoretic patterns of equal aliquots of serum from the patients and from normal subjects. The heavy lines represent normal serum, the light and broken lines represent patients' serums; reductions of γ -globulin and albumin are apparent. Beta and alpha globulins were normal or reduced except for elevated alpha globulins in patient 6, who experienced multiple infections.

Approximate Daily Rates of Disappearance of Albumin from Plasma and of Synthesis of Albumin.

TABLE III
RESULTS OF LABELLED ALBUMIN DISAPPEARANCE STUDIES IN CONTROL SUBJECTS AND PATIENTS

| Control Subjects and Patients (case no.) | Serum Albumin (gm. %) | Weight (kg.) | Half-life in Plasma (days) | Label Lost from Plasma Daily (% of total) | Label Excreted in Urine Daily (% of total) | Total Body Albumin (gm./1.73 M ²) | "Synthesis" of Albumin* (gm./day/1.73 M ²) |
|--|-----------------------|--------------|--------------------------------|---|--|---|--|
| | | | 12 10.5 ± 1.5 | 6.7 ± 0.93 | | 341 ± 47 232 ± 34 | 18.1 ± 3.2 15.4 ± 2.0 |
| Control 1 (Wilson's disease)..... | 5.0 | 71.4 | 11.8 | 5.8 | 5.0 | 366 | 21.4 |
| Control 2 (Atherosclerosis)..... | 4.5 | 71.5 | 13.0 | 5.2 | 3.5 | 271 | 14.1 |
| Control 2 (Second study)..... | 4.5 | 71.5 | 11.5 | 5.5 | 4.2 | 252 | 13.9 |
| Control 3 (Hypogammaglobulinemia)..... | 4.5 | 54.0 | 11.5 | 5.5 | 4.0 | 210 | 11.6 |
| Case I..... | 3.8 | 41.0 | 6.6 | 10.5 | 9.0 | 248 | 26.0 |
| Case II..... | 3.3 | 58.0 | 8.2 | 8.6 | 8.5 | 184 | 15.8 |
| Case III..... | 3.0 | 61.5 | 4.3 | 16.1 | 9.4 (diarrhea) | 187 | 30.2 |
| Case IV..... | 2.4 | 43.0 | 3.8 | 18.0 | 14.0 | 96 | 17.3 |
| Case V..... | 1.3 | 56.0 | 2.5 | 27.8 | 26 to 12 (diarrhea) | 87 | 24.4 |
| Case VI..... | 2.6 | 14.0 | 8.0 | 8.7 | 3 (poor collection) | 135 | 11.5 |

NOTE: Bold face figures represent normal values from the literature. Top row: Eisenmenger [16]. Bottom row: Sterling [17].

* This calculation is based on many unproved assumptions and can only be considered an approximation, especially when diarrhea is present.

Figure 6 compares the plasma disappearance curves of albumin in two patients and two normal subjects. An abnormally rapid fall in the patients' plasma radioactivity is apparent. All data on patients and control subjects are summarized in Table III. The half-lives of I^{131} albumin in normal subjects reported in studies conducted in a manner analogous to this one are given in the top row [12,16]. Next are recorded the half-lives in control subjects who were studied simultaneously with our patients, using the same albumin preparations. The half-lives of labelled albumin in these control subjects lay directly in the range reported for normal subjects, as did the calculated daily synthesis of albumin.

Under circumstances of normal catabolism of iodinated albumin, the I^{131} label is removed from the protein and excreted almost quantitatively in the urine [17]. Only small amounts are lost in the stools and perspiration. Thus the per

cent of the total body radioactivity lost per day in the urine should approximate the per cent of plasma radioactivity disappearing daily. This occurred in the control subjects.

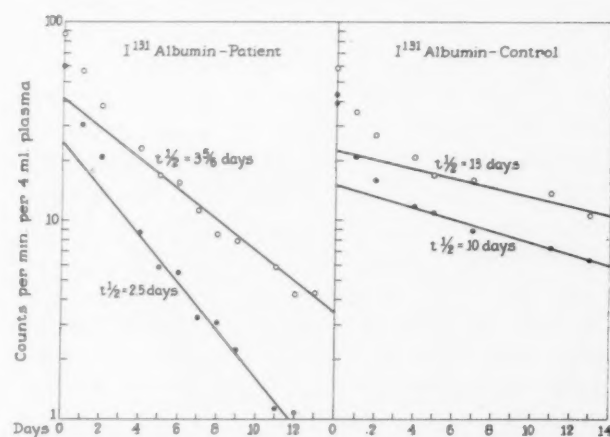


FIG. 6. Plasma disappearance curves of labelled albumin in two patients with hypoproteinemia and two control subjects.

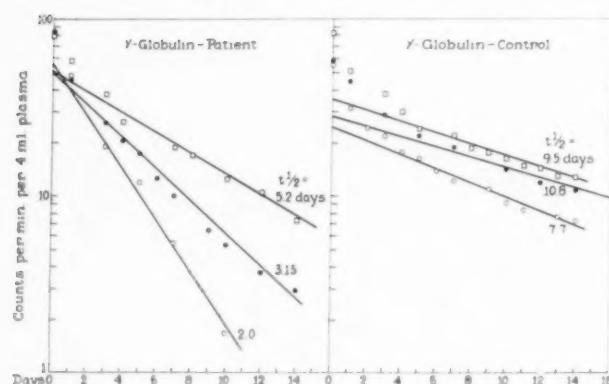


FIG. 7. Plasma disappearance curves of labelled γ -globulin in three patients with hypoproteinemia and three control subjects.

All of the patients had biologic half-lives of I^{131} albumin shorter than the control subjects; the per cent of plasma albumin disappearing daily was therefore greater than normal. The total body albumin of the patients was depressed in most instances and the calculated synthesis of albumin per day was normal or slightly elevated. This suggests that the patients lost excessive amounts of plasma albumin daily and, although they were capable of synthesizing albumin, were unable to compensate for the loss with a commensurate increase in synthesis.

However, the calculation of albumin synthesis is based upon a number of assumptions. One, that the total body albumin remained unchanged during the study was apparently accurate because both control subjects and patients maintained steady serum albumin levels and body weight and did not accumulate edema. Therefore the daily synthesis was assumed to be equal to the daily degradation. Other assumptions, for example those dealing with the distribution of albumin in body spaces and calculations of total body albumin, have never been verified. Their applicability in particular to this type of patient, especially when diarrhea is present, has never been explored. Therefore the calculated rates of synthesis must be considered approximations, perhaps varying considerably from the actual rates.

In those patients without diarrhea whose stools did not contain significant radioactivity, the per cent of total I^{131} excreted in the urine daily approximated the per cent of plasma albumin lost daily. Thus, in some way, label was removed from the albumin within the body and excreted in the urine.

Approximate Disappearance Rates of γ -globulin

TABLE IV
RESULTS OF LABELLED γ -GLOBULIN DISAPPEARANCE
STUDIES IN CONTROL SUBJECTS AND PATIENTS

| Control Subjects and Patients (case no.) | Half-life in Plasma (days) | Label Lost from Plasma Daily (%) |
|---|----------------------------------|--|
| | 10-30 | Variable |
| Control 1 (Wilson's disease) | 9.5 | 7.3 |
| Control 2 (Atherosclerosis) | 7.7 | 9.0 |
| Control 3 (Macroglobulinemia) | 12.5 | 5.5 |
| Control 4 (Biliary cirrhosis) | 8.3 | 8.4 |
| Control 5 (Atherosclerosis) | 11.5 | 6.0 |
| Control 6 (Atherosclerosis) | 12.5 | 5.5 |
| Control 7 (Agammaglobulinemia) | 9.6 | 7.2 |
| Case I | 6.3 | 11.0 |
| Case II | 6.5 | 10.6 |
| Case III | 3.8 | 18.0 |
| | 3.1 | 22.0 |
| Case IV | 5.5 | 12.6 |
| Case V | 2.0 | 34.7 |
| Case VI | 7.5 | 9.2 |

NOTE: Bold face numbers represent normal values from the literature.

from the Plasma. As mentioned previously, preparations of I^{131} γ -globulin were far less stable than albumin. Therefore, detailed calculations of the metabolism of γ -globulin by the subjects were not made because the biologic half-life of the protein could not be accurately estimated. The only data worth reporting were the comparative half-lives of the same I^{131} γ -globulin preparation in control subjects and in patients.

Figure 7 shows plasma disappearance curves for γ -globulin in three patients and three control subjects. Table IV contains all the results. Published normal human half-lives for γ -globulin obtained by this method vary from ten to thirty days. Our control values were near ten days, which emphasizes the unsatisfactory nature of the labelled γ -globulin. Nevertheless, the longest half-life found in a patient was shorter than the shortest found in a control subject studied simultaneously. Thus it is likely that the loss of γ -globulin from the patients' plasma was abnormally rapid.

TABLE V
DEMONSTRATION OF FREE AND PROTEIN-BOUND RADIOACTIVITY IN INTESTINAL JUICE OF PATIENTS
FOLLOWING INTRAVENOUS INJECTION OF LABELLED PROTEIN

| Case No. | Material Injected | Unprecipitated Juice | TCA* Supernate (immediate) | TCA Precipitate (immediate) | TCA Supernate (after 30 min. at 37°C.) | TCA Precipitate (after 30 min. at 37°C.) | Stool Radioactivity |
|----------|-----------------------------|----------------------|----------------------------|-----------------------------|--|--|---------------------|
| IV | I ¹³¹ albumin | 221 | 86 | 130 | ... | .. | 0 |
| IV | I ¹³¹ albumin | 147 | 58 | 47 | 143 | 0 | 0 |
| V | I ¹³¹ albumin | 211 | 111 | 100 | ... | .. | + |
| V | I ¹³¹ albumin | 74 | 55 | 30 | 64 | 0 | + |
| IV | I ¹³¹ γ-globulin | 232 | 172 | 11 | ... | .. | ± |
| V | I ¹³¹ γ-globulin | 124 | 77 | 0 | ... | .. | + |

NOTE: Numbers represent counts per minute of certain volume of intestinal juice. Experiments were conducted so that each horizontal set of figures were obtained from aliquots of one sample of juice. Sum of supernate and precipitate counts should equal that of unprecipitated juice.

* Trichloroacetic acid.

Recovery of Intravenously Injected Proteins from Intestinal Secretions. During the course of albumin degradation studies in three patients and γ-globulin degradation studies in two, samples of jejunal fluid were collected. This fluid was consistently radioactive. In patient 2, collection was difficult and "washing" through the tube with saline solution was necessary. Although the amount of radioactivity recovered was not large, it was distinct. With patients 4 and 5, adequate collections of intestinal fluid were possible and the results are summarized in Table v.

When fresh fluid was precipitated with TCA immediately upon withdrawal from the intestine as much as 60 per cent of the radioactivity could be found in the precipitate and was therefore bound to protein (presumably the original albumin). On the other hand, if another aliquot of the same fresh fluid were allowed to stand at 37°C. for thirty minutes before TCA was added, all the radioactivity was found in the supernate. Thus the ability of intestinal enzymes to digest the label from the protein was established. It therefore appears that serum proteins pass into the intestine and are digested by intestinal enzymes. In the experiments with I¹³¹ proteins the label was presumably removed enzymatically, reabsorbed from the lumen, and then excreted by the kidney. This explains the recovery of label from the urine despite the fact that the serum protein is not "catabolized" in the true sense of the word, but rather digested in the intestinal lumen.

A corollary of the ability of the enzymes to

digest the proteins is the fact that protein-bound radioactivity will be found in intestinal fluid only if that fluid is of such recent origin that enzymes did not have an adequate period to destroy the proteins before the sample is withdrawn. This requirement may provide the explanation for the failure to recover more than negligible amounts of protein-bound I¹³¹ in intestinal juice in the experiments in which I¹³¹ γ-globulin was given intravenously.

Immunological Identification of Serum Proteins in Intestinal Fluids. Fresh, frozen intestinal juice was thawed at 4°C. as described. Supernates from centrifugation were examined for the presence of serum proteins by capillary tube precipitation and agar diffusion technics. Reactions were obtained by both methods between the intestinal fluid from patients and the antisera to whole normal human serum, normal human albumin, and normal human γ-globulin. Figure 8 shows an agar diffusion plate with intestinal juice from Case v. The juice forms single merging bands with albumin or γ-globulin when diffusing against antisera to these proteins. Numerous additional bands are present between the intestinal fluid and the antisera to whole serum, indicating the presence of more than two serum proteins.

The identification of serum proteins in intestinal fluid was not limited to the hypoproteic patients; both immunologic methods showed serum proteins to be present in the fluid from a variety of normal and control subjects. Figure 9A is an agar plate of intestinal fluid from a person



FIG. 8. Agar diffusion plate with intestinal fluid from patient 5. Intestinal fluid is in wells 6 and 7. Well 1 contains human serum albumin 1 mg./ml. Well 3 contains antiserum to human serum albumin. Well 2 contains human γ -globulin 1 mg./ml. Well 5 contains antiserum to human γ -globulin. Well 4 contains antiserum to whole normal human serum.

with normal serum proteins and no demonstrable intestinal disease. A number of serum proteins can be identified.

The ability of intestinal enzymes to digest the serum proteins is again indicated in Figure 9B. The intestinal fluid of 9A was thawed in the presence of trypsin inhibitor. An aliquot of the same sample of fluid was thawed and allowed to stand 30 minutes at 37°C. before trypsin inhibitor was added, and the fluid again chilled. This was used in the plate 9B. The albumin band has disappeared, as have those of some other serum proteins. In all experiments γ -globulin appeared to resist intestinal enzyme digestion better than other serum proteins.

Serum proteins were also found in saliva and gastric juice from normal persons and from the



FIG. 10. Agar diffusion plate of normal human saliva concentrated five times. Wells 6 and 7 contain saliva. Well 1 contains human albumin 1 mg./ml. Well 3 contains antiserum to human albumin. Well 2 contains human γ -globulin 1 mg./ml. Well 5 contains antiserum to human γ -globulin. Well 4 contains antiserum to whole normal human serum.

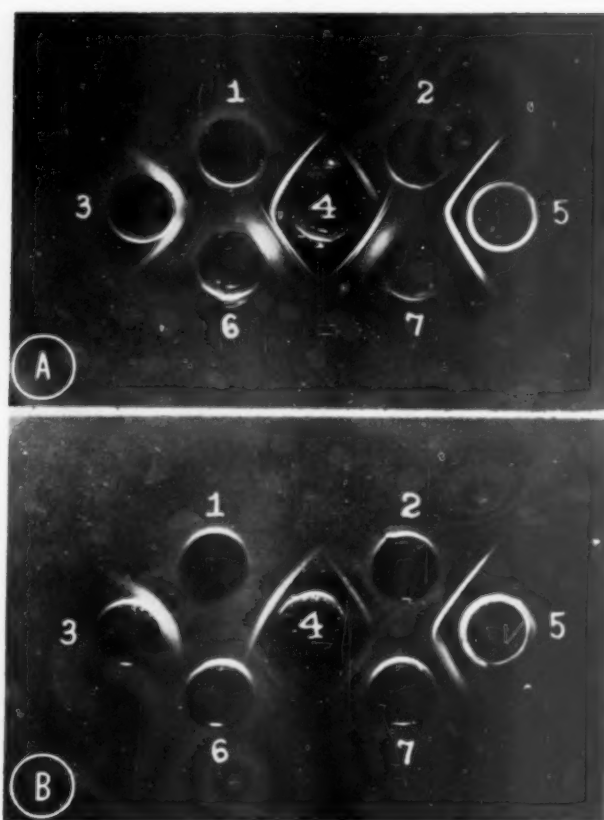


FIG. 9. Agar diffusion plate of intestinal fluid from a normal person. A, fluid kept at 4°C. with trypsin inhibitor present. B, sample of same fluid kept thirty minutes at 37°C. before trypsin inhibitor added. Wells 6 and 7 contain intestinal fluid. Well 1 contains human albumin 1 mg./ml. Well 3 contains antiserum to human albumin. Well 2 contains human γ -globulin. Well 5 contains antiserum to human γ -globulin. Well 4 contains antiserum to whole normal human serum.

patients under study. Figure 10 is an agar plate of saliva from a normal person concentrated to a fifth of its original volume. The presence of albumin and γ -globulin is evident. Stimulation of gastric secretion with histamine diminished the concentration of serum protein in the gastric juice. While this might have been an enzymatic effect, it is possible that it was merely due to dilution. It is likely that a portion of the serum protein in gastric juice is derived from swallowed saliva. It is unlikely that saliva is a significant source of serum protein in intestinal fluid, however, because of the long exposure of saliva to enzymes while enroute to the intestine and because the proteins are present in higher concentration in intestinal juice than in saliva (Figures 8 and 9 are of unconcentrated intestinal fluid, while Figure 10 is of saliva concentrated five times).

It was not possible to estimate the comparative

amounts of serum proteins lost into the intestine daily by normal and hypoproteinemic subjects, because the volumes of intestinal fluid were not known, and enzymatic digestion of the proteins occurs. In general, it was easier to get samples of intestinal fluid containing serum proteins, and to have the proteins in higher concentration, when aspirating the hypoproteinemic patients than when aspirating normal persons. However, some samples from normal subjects appeared to contain as much serum protein as samples from patients, as evidenced by the fluid in Figure 9A.

COMMENTS

The use of albumin labelled with radioactive iodine to determine plasma disappearance rates appears acceptable in these experiments. Immunologic, electrophoretic and ultracentrifugal analysis, and plasma disappearance curves after injection into rabbits, all have shown I^{131} albumin to be indistinguishable from unlabelled human albumin [12,17]. Negligible amounts of the label have been found to be unbound and the label has not been transferred to other serum proteins or reutilized in other known ways. In the present experiments the labelled albumin migrated as albumin in electrophoresis and only the albumin of the patient's serum proteins was found to contain label. Less than 2 per cent of the radioactivity of the injected albumin was dialyzable or not precipitable by TCA, and the same was true of plasma radioactivity during the study.

Yalow and Berson have shown that radioiodinated albumin is altered by self-radiation in such a way as to reduce its biologic half-life, and that this effect could be minimized by adding normal albumin to the labelled material [18]. This and other considerations led them to regard radioiodinated serum albumin as an unsatisfactory material for measuring absolute biologic half-lives of albumin [17]. While concurring with this view, we do not believe that these shortcomings apply to this study. The iodinated proteins were usually used immediately upon receipt, and usually with carrier albumin. Furthermore, no effort was made to measure the absolute biologic half-life in the patients. Rather, the disappearance rate from plasma was measured and compared with that of a control subject studied simultaneously, using the same preparation of albumin.

For these same reasons, despite the serious

inadequacies of the γ -globulin preparations, the differences between the disappearance rate obtained in the patients as compared with the control subjects suggest that an excessive loss of protein from the plasma is responsible for the low serum levels.

The recovery of TCA precipitable radioactivity and of immunologically identifiable albumin and γ -globulin from the intestinal fluids suggested that loss of serum protein into the intestinal lumen might be the cause of the rapid disappearance from plasma. However, the identification of many different serum proteins in the intestinal secretions of normal persons indicates that this mechanism, if it is responsible for the hypoproteinemia, represents only a quantitative rather than a qualitative departure from normal.

Measurement of PVP excretion is the best method currently available for quantitative estimation of intestinal protein loss. Gordon has found a small stool loss of PVP introduced intravenously in normal subjects and control subjects, and a somewhat larger loss in patients with intestinal diseases or idiopathic hypoproteinemia. This is compatible with the identification of serum proteins by immunological methods in the intestinal fluid of similar groups of people. In his study the increase above normal of PVP excretion in the stool in idiopathic hypoproteinemia varied from 50 per cent to 800 per cent. In our cases the increase of albumin turnover per day varied from 50 per cent to 500 per cent above normal. However, it remains only a presumption that PVP and serum proteins enter the intestine in the same manner.

When diarrhea is present, loss of serum proteins or amino acids from the body via the stools could play a major role in the hypoproteinemia. However, when diarrhea is absent, as in some of these cases, the hypoproteinemia would then depend upon excessive loss of protein into the intestine, digestion of the protein, and failure of resynthesis of a commensurate amount of serum protein. The calculated total body albumin synthesis in the patients appears to be normal. This is true despite the fact that patients without diarrhea apparently resorb the breakdown products of the serum proteins. Thus, as in nephrosis [16], there appears to be an inability to increase albumin synthesis when confronted with an excessive loss of albumin.

The present data show that serum proteins

disappear from the patients' serums more rapidly than normal, that they are present in the intestinal fluid and can be digested by intestinal enzymes, and that the patients' daily synthesis of albumin is probably normal. They do not demonstrate that the loss into the intestine is greater than normal or that it is responsible for the hypoproteinemia. The striking disappearance of hypoproteinemia after resection of diseased bowel in two cases indicates that the intestine was the site of protein loss. Nevertheless, more effective technics will be necessary in order to establish that excessive loss of protein into the intestine occurs, and to determine whether this is the sole abnormality, or whether abnormalities of digestion, absorption, or synthesis of protein also exist.

Current data do not permit a decision on the question of whether serum proteins are actively secreted into the intestine or appear by passive transudation. Injection of histamine or secretin failed to increase the identifiable serum protein in the gastric or intestinal juices, respectively. This suggests that the proteins are not secreted, although it may reflect only a simultaneous increase in enzymatic activity. PVP is presumably inert and its passage into the intestine might more likely be due to a passive process than an active secretion. However, glandular hypersecretion may exist in these patients and protein may not be the sole serum constituent secreted in excessive amounts. The hypertrophied rugae in patients with hypertrophic gastritis and hypoproteinemia would be compatible with this possibility.

It is interesting that in these patients hypoproteinemia appeared before evidence of significant intestinal disease. The loss of serum protein into the intestine in typical cases of regional enteritis and ulcerative colitis might be explained solely by exudation at the site of mucosal inflammation; the presence of hypoproteinemia prior to development of specific intestinal lesions suggests that an abnormality exists which is more than simple exudation. In fact, excessive loss of serum protein into the intestine may prove to be a harbinger of future intestinal lesions.

The physiological significance of the appearance of serum protein in normal intestinal fluid is obscure. However, knowledge of the mechanism of destruction of serum proteins in the body is incomplete. Possibly digestion by intestinal enzymes is one such mechanism.

The finding of normal serum lipids and cholesterol in these patients is interesting. It appears to be in contradiction with the hypothesis, derived from studies in nephrosis, that hypercholesterolemia is a direct consequence of hypoalbuminemia.

Clinically, several points merit mention. Case vi appears to be one of primary hypogammaglobulinemia with subsequent development of an intestinal lesion resulting in hypoalbuminemia. After removal of diseased bowel the serum albumin level returned to normal but hypogammaglobulinemia persisted. It is of interest in this connection that unusual intestinal diseases have occurred in association with agammaglobulinemia, presumably due to intestinal infections [19]. The significance of the thickening of arterial intima in the intestinal wall in this patient is not clear, for there was no evidence of generalized polyarteritis.

Cases i and ii may represent forms of regional enteritis, although neither clinical course nor pathological findings are typical, for pain, fever and weight loss were not prominent. Cases iii, iv and v present minimal or no demonstrable abnormality of the intestine. This suggests that excessive loss of protein into the intestine may be present without specific morphologic lesions and thus may be associated with a variety of intestinal disorders.

The pathologic picture in these patients is not uniform and permits no specific classification. Dilatation of mesenteric lymphatics was pronounced in Cases i and ii. Increased pressure within lymphatics might contribute to protein loss across the intestinal mucosa. However, lymphatic obstruction was not present in all cases.

Responses to therapy varied. In two cases excision of diseased bowel resulted in striking improvement despite the fact that not all areas of inflamed mesentery or bowel were resected. In three of the six patients either restriction of fat intake or administration of corticosteroids was beneficial for limited periods. In two cases no therapy was truly satisfactory.

The nature of the anemia these patients demonstrate is not clear. Serum iron and iron-binding protein are usually normal. The marrow is only slightly hyperplastic; peripheral red cells are mildly hypochromic. This anemia may be similar to that of experimental animals undergoing plasmaphoresis who apparently divert hemoglobin to plasma protein synthesis [20].

SUMMARY

1. Six patients are reported with "idiopathic" hypoproteinemia and rapid loss of albumin and γ -globulin from the plasma. The appearance of hypoproteinemia antedated the development of intestinal lesions in three of these patients.

2. Albumin and γ -globulin have been demonstrated in the intestinal juice of these patients and of normal persons. The possibility exists that the hypoproteinemia is a consequence of excessive loss of serum protein into the intestine.

3. Experiences with treatment are presented. Two patients improved greatly after resection of diseased intestine.

4. Although all the intestinal lesions appeared to be inflammatory, no specific pathological classification was possible.

REFERENCES

1. HOMBURGER, F. and PETERMANN, M. Familial idiopathic dysproteinemia. *Blood*, 4: 1085, 1949.
2. BENNHOLD, H., PETERS, H. and ROTH, E. Congenital dysproteinemia. *Verhandl. deutsch. Gesellsch. inn. Med.*, 60: 630, 1954.
3. ULSTROM, R., SMITH, N. and HEIMLICH, E. Transient dysproteinemia in infants. *Am. J. Dis. Child.*, 93: 536, 1957.
4. ALBRIGHT, F., FORBES, A., BARTTER, F., REIFENSTEIN, E., BRYANT, D., COX, L. and DEMPSEY, E. Studies on the Fate of Intravenously Administered Human Plasma Proteins in Idiopathic Hypoproteinemia and in Osteoporosis. *Plasma Proteins. Symposium on Nutrition*, vol. 2. Springfield, Ill., 1950. Charles C Thomas.
5. SCHWARTZ, M. and THOMSEN, B. Idiopathic and hypercatabolic hypoproteinemia. *Brit. M. J.*, 1: 14, 1957.
6. STEINFELD, J., DAVIDSON, J. and GORDON, R. A mechanism for hypoalbuminemia in patients with ulcerative colitis and regional enteritis. *J. Clin. Invest.*, 36: 931, 1957.
7. CITRIN, Y., STERLING, K. and HALSTED, J. The mechanism of hypoproteinemia associated with giant hypotrophy of the gastric mucosa. *New England J. Med.*, 257: 905, 1957.
8. GORDON, R. Exudative enteropathy. *Lancet*, 1: 325, 1959.
9. SCHWARTZ, M. and JARNUM, S. Gastrointestinal protein loss in idiopathic (hypercatabolic) hypoproteinemia. *Lancet*, 1: 327, 1959.
10. GORDON, R., BARTTER, F. and WALDMANN, T. Idiopathic hypoalbuminemia; clinical staff conference at the National Institutes of Health. *Ann. Int. Med.* (In press.)
11. STERLING, K. The turnover rate of serum albumin in man as measured by I^{131} -tagged albumin. *J. Clin. Invest.*, 30: 1228, 1951.
12. HIRSCH, J., AHRENS, E. and BLANKENHORN, D. Measurement of the human intestinal length in vivo and some causes of variation. *Gastroenterology*, 31: 274, 1956.
13. OUCHTERLONY, O. Antigen-antibody reactions in gels. IV. Types of reactions in coordinated systems of diffusion. *Acta path. et microbiol. Scandinav.*, 32: 231, 1953.
14. KUNKEL, H. G. and TISELIUS, H. Electrophoresis of proteins on filter paper. *J. Gen. Physiol.*, 35: 89, 1951.
15. SHINER, M. Duodenal and jejunal biopsies. *Gastroenterology*, 33: 64, 1957.
16. EISENMENGER, W. Hepatic function and protein metabolism in cirrhosis of the liver. *M. Clin. North America*, 39: 719, 1955.
17. BERSON, S., YALOW, R., SCHREIBER, S. and POST, J. Tracer experiments with I^{131} labeled human serum albumin: Distribution and degradation studies. *J. Clin. Invest.*, 32: 746, 1953.
18. YALOW, R. and BERSON, S. Chemical and biological alterations induced by irradiation of I^{131} labeled human serum albumin. *J. Clin. Invest.*, 36: 44, 1957.
19. SANFORD, J., FAVAR, C. and TRIGEMAN, M. Absence of γ -globulins in an adult. *New England J. Med.*, 250: 1027, 1954.
20. MADDEN, S., KATTUS, A., CARTER, J., MILLER, L. and WHIPPLE, G. Plasma protein production as influenced by parenteral protein digests, very high protein feeding, and red blood cell catabolism. *J. Exper. Med.*, 82: 181, 1945.

Seminar on Mycotic Infections

Cryptococcosis (Torulosis)*

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ONCE considered to be a rare disease, cryptococcosis is now being recognized more frequently both as a primary and a secondary infection in man. The causative agent of the disease is *Cryptococcus neoformans*, a pathogenic yeast that resides in the soil as a saprophyte. It is found also in great numbers in pigeon excreta. Inhalation of cryptococcus-laden pigeon excreta dust by susceptible persons may cause primary pulmonary cryptococcosis, a benign and usually self-limited respiratory disease. Disseminated cryptococcosis, which invariably is fatal if untreated is more prone to develop in persons with low host resistance or reticulo-endothelial diseases, such as leukemia, malignant lymphoma or Hodgkin's disease.

Dissemination of *C. neoformans* occurs from foci in the lungs via the blood stream to all organ systems, including the central nervous system. Once the yeast has broken through the blood-brain barrier and has seeded the spinal fluid it is dispersed quickly over the surfaces of brain and spinal cord by the cerebral circulation of spinal fluid. Within the central nervous system the parasite finds the nutrients thiamin, glutamic acid and certain carbohydrates in optimal concentrations for growth and for production of large capsules [1]. Elaboration of capsular substance by the organism further increases its resistance to the normal defense mechanisms of the host. The meninges react to the presence of the yeast and its metabolic products with the formation of obstructive leptomeningitis and intracranial block, resulting in elevated hydrostatic pressure of the spinal fluid and development of internal hydrocephalus. As the disease progresses the living yeast cells metabolize

more and more of the carbohydrates and carbohydrate derivatives of the spinal fluid. The protein content of the spinal fluid increases, cells become more numerous, and the sugar content diminishes.

When the organism enters the central nervous system, prognosis of the untreated case becomes grave. A patient with fulminant cryptococcal meningoencephalitis may succumb within two weeks but the usual duration of the untreated fatal illness is approximately four to six months. Chronic infections have been known to persist for years and, if untreated, also terminate fatally. An effective intravenous medication for the treatment of disseminated cryptococcosis is amphotericin B (Fungizone®), a polyene antibiotic [2-8]. This antibiotic has limited value, however, for the treatment of fungal meningitides, since only barely discernible levels of the antibiotic or none at all are produced in the spinal fluid following intravenous administration, and only minute amounts of amphotericin B can be administered safely by intrathecal route. Nevertheless, the severity of the meningitis and the resultant mortality are lessened when patients are treated with this medication.

CLINICAL AND PATHOLOGIC FEATURES

A comprehensive description of the clinical, pathological and microbiological features of cryptococcosis may be found in the 1956 monograph by Littman and Zimmerman [9]. Some of the salient characteristics of the disease are described herein.

Pulmonary Cryptococcosis. Pulmonary cryptococcosis occurs without involvement of the nervous system. However, the signs and symp-

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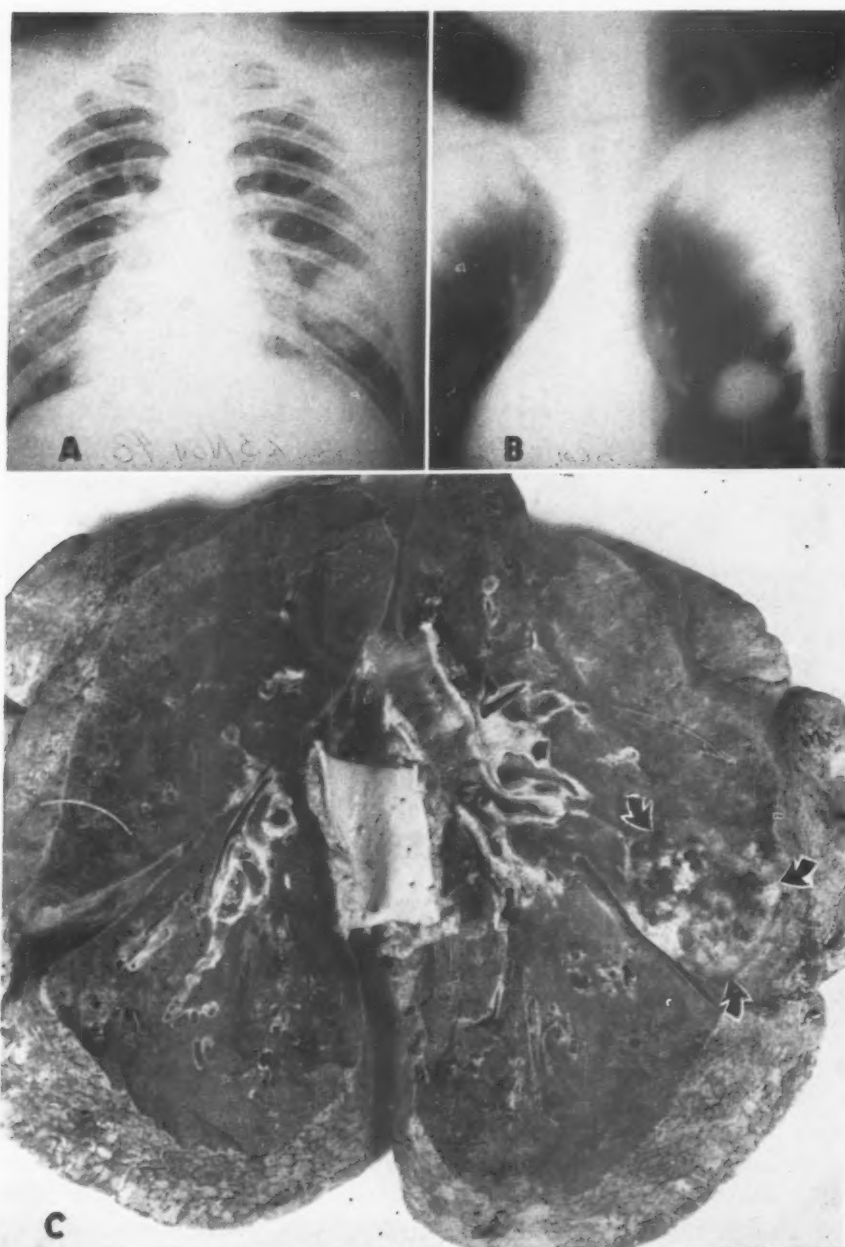


FIG. 1. Discrete pulmonary granuloma due to *C. neoformans*, a fatal case of cryptococcal meningoencephalitis. Two and a half months after admission the patient noted headache, meningoencephalitis developed and he died one month later. A, (posteroanterior view) discrete but not sharply circumscribed lesion in right lower lung field. B, same lesion demonstrated by laminograph. C, lungs at autopsy sectioned longitudinally and posterior halves removed. The pulmonary granuloma lies beneath the pleura but occupies much of the middle lobe of the right lung. The pleural reaction is minimal. Case reported by Ratcliffe and Cook [70]. (From: Littman, M. L. and Zimmerman, L. E. Cryptococcosis. New York, 1956. Grune & Stratton.)

toms of pulmonary infection are not characteristic and many pulmonary cases may be asymptomatic. When present, the symptoms of cryptococcal pulmonary infection include cough, scanty mucoid sputum and infrequent hemoptysis. There may be low grade fever, pleuritic

pain and weight loss but these symptoms are rarely prominent. Night sweats seldom occur.

A specific diagnosis of cryptococcal pulmonary infection may be established by culture of the sputum or bronchoscopic aspirate, or by bronchoscopic biopsy. Since *C. neoformans* is

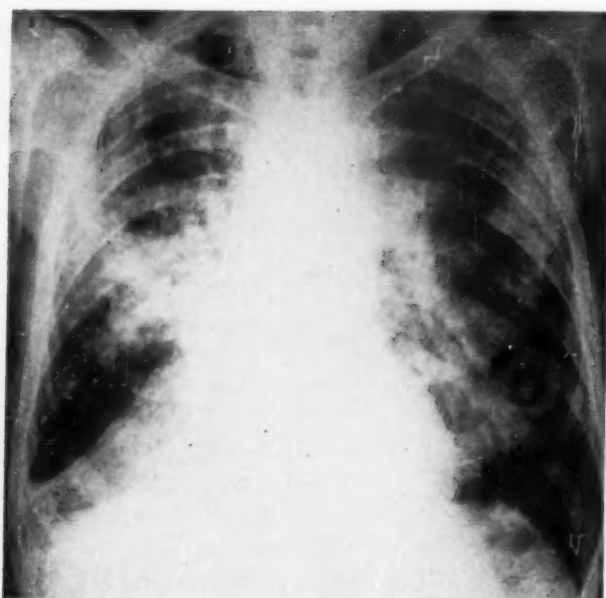


FIG. 2. Extensive bilateral pulmonary cryptococcosis simulating far advanced tuberculosis. (From: Littman, M. L. and Zimmerman, L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

normally absent from the sputum, isolation of this organism is of diagnostic significance.

The appearance of chest roentgenograms of patients with pulmonary cryptococcosis varies considerably. The most common lesion is a solitary, moderately dense area of infiltration in the lower half of either lung field, varying in size from 2 to 7 cm. [9,10]. (Fig. 1.) Coin lesions rarely are observed in pulmonary cryptococcosis and there is little or no hilar enlargement. Diffuse, broad pneumonic infiltrations may occur, with accentuation of bronchovascular markings and small nodular shadows. Most patients show minimal pleural reaction. Organizing bronchopneumonia is unusual but has been described. Caseation necrosis and cavitation seldom occur, but have been described in two cases [11]. Widespread minute lesions of the lungs, indistinguishable from miliary tuberculosis and those simulating far advanced tuberculosis, are often encountered in patients with malignant lymphoma and disseminated cryptococcosis. (Fig. 2.) Thus, judging from the marked roentgenologic variations in pulmonary cryptococcosis, it is necessary to include cryptococcosis in the differential diagnosis of pulmonary diseases of slow evolution, such as primary and secondary carcinoma, sarcoidosis, chronic pulmonary tuberculosis, bronchiectasis, pneumoconiosis, hydatid cyst, and fungus infections other than cryptococcosis. Important radiologic findings

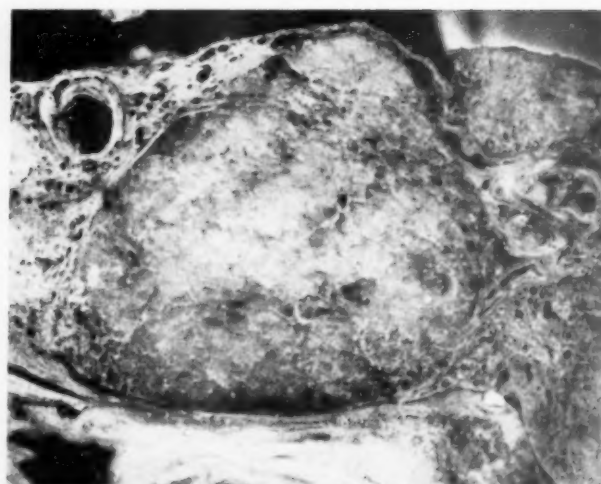


FIG. 3. Myxomatous character of cryptococcal lesion of the lung (approximate actual size). (From: Littman, M. L. and Zimmerman L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

characteristic of pulmonary cryptococcosis are a predilection for the lower half of the lung fields, rare cavitation, minimal or absent fibrosis or calcification, inconspicuous hilar lymphadenopathy and infrequency of massive pulmonary collapse [9].

At autopsy, gross examination of the lungs may reveal either widespread involvement of tissues or a localized process. Localized solitary cryptococcal masses in the lung, ranging from 2 to 7 cm., may be found at either the lung periphery close to the hilum, or in the mid-area of a lobe. If the number of cryptococci in the tissue is small and the tissue reaction is marked, the masses may appear firm and rubbery on gross examination, giving a non-specific granulomatous appearance. On the other hand, if there are massive accumulations of cryptococci the pulmonary masses may be mucoid in appearance [9]. (Fig. 3.) Small solitary granulomas, either peripheral or subpleural, rarely excite significant pleuritis. Tissue necrosis is minimal, even in large cryptococcal granulomas. This is in marked contrast to the coagulative necrosis characteristic of coccidioidomycosis and histoplasmosis, and the microabscess formation in North American blastomycosis [9].

Disseminated pulmonary cryptococcosis is rarely encountered as a purely respiratory disease. It is more often associated with widespread involvement of other tissues in the presence of reticuloendothelial disease. Gross examination of the lungs in disseminated cryptococcosis reveals miliary tubercles resembling those of

tuberculosis [12] (Fig. 4) but which appear more mucoid than tubercular lesions on close examination.

Central Nervous System Cryptococcosis. Nervous system involvement begins insidiously without prodromal symptoms. The first complaint is usually headache, intermittent at onset, but becoming continuous and progressively severe. The pain appears most frequently in the frontal or temporal regions, least frequently over the occipital area. A more severe form of headache is associated with widespread involvement of cerebral tissue. Diplopia and photophobia are common early symptoms. Other ocular disorders are neuroretinitis, retinal hemorrhages, strabismus, nystagmus, anisocoria, ptosis, loss of pupillary reactivity to light, retinal exudates, primary optic nerve atrophy and ophthalmoplegia. Moderate to marked papilledema, due to increased spinal fluid pressure, is observed in at least half the cases. The author has encountered an increasing number of cases of cryptococcal meningitis among psychiatric patients whose chief complaints are headache. This stresses the necessity of including spinal fluid culture in the study of all psychiatric patients who give a history of such complaints.

General physical examination of the patient reveals a primary disturbance of the central nervous system and suggests meningitis or the presence of an expanding intracranial lesion. Nuchal rigidity is frequently present and Kernig's and Brudzinski's signs are often positive. Neither lymphadenopathy nor hepatosplenomegaly is a common finding, and autopsy of fatal cases usually fails to reveal significant enlargement of internal organs despite widespread dissemination of the fungus. A low grade fever of 101 to 102°F. is usually present. In the more advanced state of cerebral disease, patients become apathetic and confused, then semi-comatose. They may have difficulty in swallowing, and suction may be required to remove accumulated pharyngeal secretions. In almost every instance lumbar punctures reveal elevated pressures, and spinal fluids that appear to be clear, turbid or xanthochromic depending upon the stage and severity of the disease. Hydrostatic pressures of 150 to 300 mm. of water are more commonly encountered, but pressures up to 700 mm. have been recorded. The protein content of the spinal fluid is usually elevated and the sugar is decreased, often to 10 or 20 mg. per cent. Return of sugar values to normal as well as



FIG. 4. Miliary cryptococcal lesions of the lung in a sixty-five year old Negro man with chronic lymphatic leukemia. The lesions were composed almost entirely of cryptococci with negligible surrounding tissue reaction. Case of Baker and Haugen [12]. (From: Littman, M. L. and Zimmerman, L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

decrease in hydrostatic pressure are useful parameters of clinical improvement.

The differential diagnosis in cryptococcosis of the nervous system depends upon whether cranial involvement is diffuse or localized. Diffuse cryptococcal meningitis often resembles tuberculous meningitis, and spinal fluid changes in both diseases may be indistinguishable except by cultural means. In many cases, if the chest roentgenograms suggest tuberculosis a diagnosis of tuberculous meningitis is made. However, cryptococcal and tuberculous meningitis may be differentiated from each other by (1) the shorter and more acute clinical course of tuberculous meningitis, (2) the greater frequency of tuberculous meningitis in children, (3) the presence of proved tuberculous foci in the lungs and lymph nodes, and (4) the isolation of the causative organisms in the spinal fluid. The shorter duration and more benign course of lymphocytic choriomeningitis differentiates it from tuber-

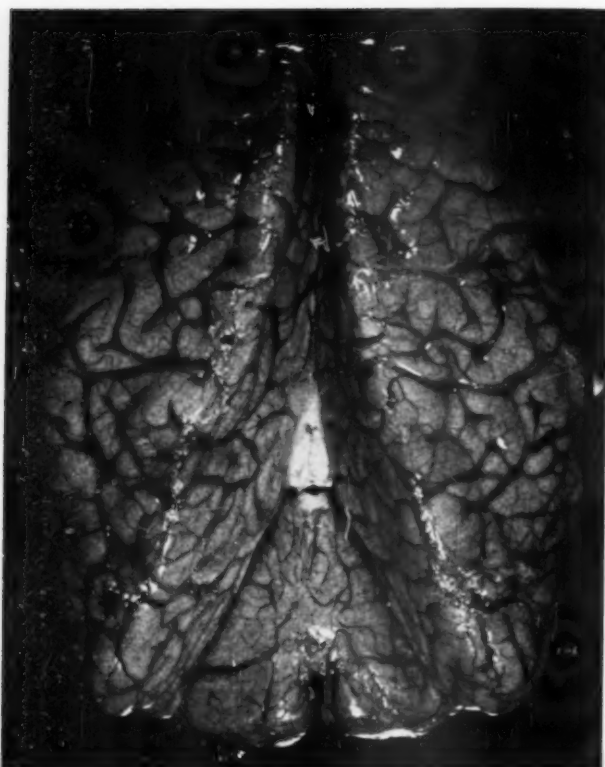


FIG. 5. Cryptococcal meningitis. Intense congestion of subarachnoid vessels but minimal exudate over cerebral hemispheres. Cerebellar convolutions are obscured by membranous exudate in the subarachnoid space. Meningeal reaction is densely granulomatous. (From: Littman, M. L. and Zimmerman, L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

culous meningitis. Neurosyphilis is readily differentiated by the Wassermann reaction of the spinal fluid. Neurological manifestations observed in cryptococcal meningitis are often interpreted as carcinomatous involvement of the meninges if the chest roentgenogram suggests carcinoma.

In cases of localized cryptococcal granuloma of the brain or cord which produce symptoms and signs suggestive of brain tumor, abscess, subdural hematoma or uncinat hernia, spinal fluid cultures may fail to reveal cryptococci unless large volumes of fluid are examined. Neural cryptococcal granulomas may form without concomitant meningitis, and on roentgenographic investigation may appear to be expanding. In such instances neurosurgical procedures may be carried out, but they bring only a brief interval of symptomatic relief to the patient and fail to alter the ultimate fatal course of the disease. Often the correct etiological diagnosis is made only after examination of biopsy material by pathological and cultural means. In cases

other than granuloma, advanced cryptococcosis of the nervous system may also simulate an expanding intracranial lesion. Roentgenographic films of the skull in these cases may reveal convolutional markings, suture separations, atrophy of dorsum sellae and clinoids, displacement of pineal gland and choroid plexus, and destruction of bone [13], all of which may complicate diagnosis.

PATHOLOGICAL CHANGES IN NERVOUS SYSTEM CRYPTOCOCCOSIS

Meningitis. Gross examination of the brain and cord of fatal cases of cryptococcal meningitis usually reveals only minimal changes. A diffuse or patchy exudate is found over the base of the brain and cerebellum (Fig. 5.) and in the spinal subarachnoid space. The leptomeninges over the cerebral hemispheres often display only minimal gross changes such as hyperemia. Exudates vary from delicate opacities to dense masses of adherent creamy white material. If the organisms are numerous and heavily encapsulated, the exudate may become relatively transparent and amber colored. The subarachnoid space is often distended by an adherent exudate, in which case the membrane can be easily lifted. Histological examination of the leptomeninges reveals the granulomatous meningitis, resembling that seen in tuberculous meningitis and in other fungal meningitides. The degree of reaction in the meninges varies from one area to another but is generally characterized by chronic inflammation, angiitis and formation of granulation tissue. Cellular reaction in the subarachnoid space may be minimal around the foci of organisms, whereas an adjacent field may show a prominent histiocytic response, with numerous phagocytized organisms contained within mononuclear inflammatory cells. Giant cells are invariably present, however. Densely granulomatous meninges usually contain only small numbers of organisms, revealed by special stains for mucin or by Gomori's methenamine-silver nitrate stain. Although the parasite usually invokes a macrophagic, cellular response, exudation of polymorphonuclear leukocytes may occur [9]. The latter is seldom of such degree as to lead to confusion with acute pyogenic bacterial meningitis.

Meningoencephalitis. In addition to meningeal invasion, cryptococcal infection may extend along perivascular sheaths into the brain sub-

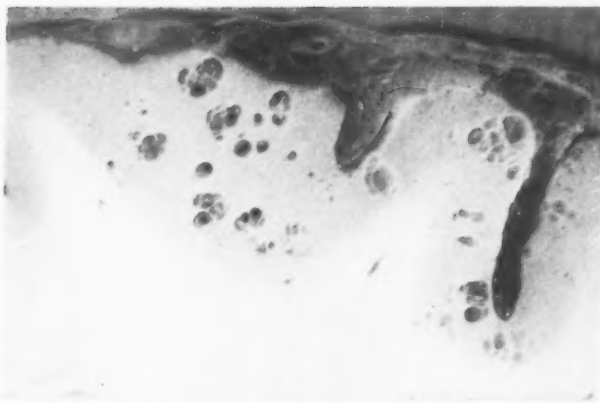


FIG. 6. Cryptococcal meningoencephalitis. Multiple minute cystoid spaces filled with cryptococci and capsular polysaccharide are distributed along the cerebral gray matter. There is no wall or edema. (From: Littman, M. L. and Zimmerman, L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

stance, causing diffuse involvement of the brain and meningoencephalitis. Proliferation of organisms at perivascular sites causes irregular studing of the gray matter with tiny but grossly visible cysts. In addition to the changes observed in the leptomeninges, there may also be a honeycomb appearance of the cerebral cortex, basal ganglia, cerebellar nuclei and brain stem, confined primarily to gray matter. (Fig. 6.) *C. neoformans* is not proteolytic and the "honeycombed" or "soapsuds" appearance of the cysts, seen on gross examination of the gray matter, represents expansion of fungus colonies *in vivo* into adjacent tissue rather than dissolution of brain tissue. Section of fresh infected brain tissue exposes the glary, mucoid material of the tiny cysts, which are composed of accumulated capsular polysaccharides of colonies of cryptococci. Formalin fixation causes shrinkage of the mucoid cyst contents and accounts for the empty, cyst-like appearance of the lesions in fixed specimens. (Fig. 6.)

In meningoencephalitis, the meninges usually show little evidence of inflammation. Cryptococci exist in abundance in the subarachnoid space. Virchow-Robin spaces in the involved areas of the parenchyma are filled with cryptococci and are often greatly distended by submicroscopic colonies of cryptococci. Cystic lesions frequently communicate with the subarachnoid space by narrow perivascular zones of involvement. Their rupture into the subarachnoid space undoubtedly accounts for periodic seeding of the spinal fluid with cryptococci. (Fig. 7.) Gross examination of cysts reveals



FIG. 7. Cryptococcal meningoencephalitis. Subarachnoid space is markedly distended with cryptococci and capsular polysaccharide. There is perivascular extension of infection into cerebral parenchyma as well as communication with subarachnoid space. (From: Littman, M. L. and Zimmerman, L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

no evidence of a wall or edema, and histological examination usually shows an absence of cellular reaction. Injury to the parenchyma is undoubtedly brought about by pressure of cryptococcal masses upon peripherally adjacent tissue. Parenchymatous lesions in the brain also may be produced by a process of embolism, most easily recognized in deep-seated lesions. Collections of cryptococci are almost certain to be embolic when found in periventricular and periaqueductal gray matter, in the basal ganglia, dentate nucleus of the cerebellum, in white matter of the cerebral hemisphere, and beneath the ependyma [9].

Granuloma. Cryptococcal infection of the nervous system may be limited to well demarcated granulomas which resemble gummas of the cerebrum, cerebellum or cord. These may cause obstruction anywhere in the ventricular system, giving rise to internal hydrocephalus. Filling defects may be caused by projection of miliary granulations or large masses into the ventricles from subependymal gray matter or from choroid plexus [9]. On the other hand, if lesions are predominantly meningeal, external

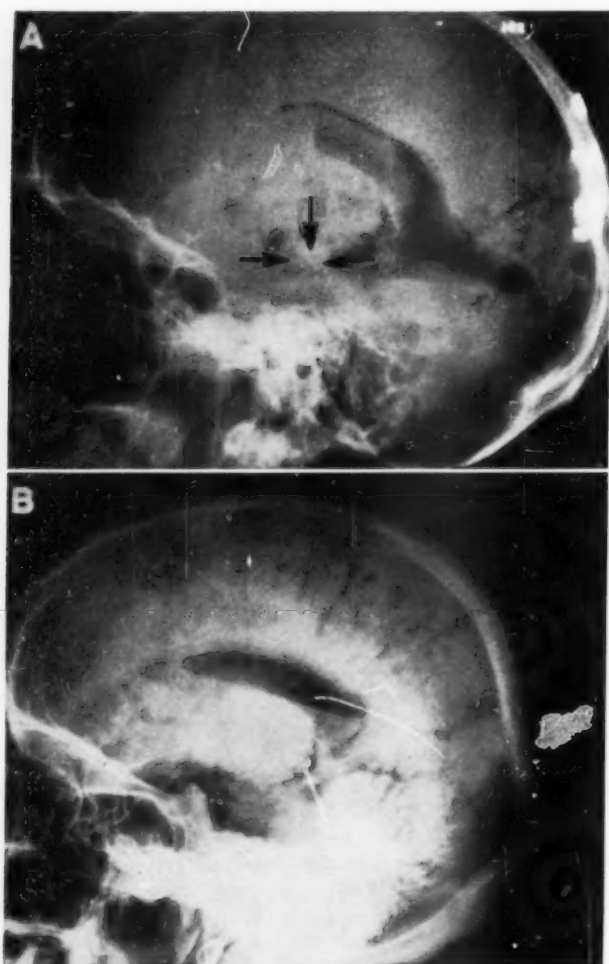


FIG. 8. Cryptococcal meningitis simulating neoplasm of the brain stem. The ventriculoencephalogram A, shows an obtuse angle of the aqueduct of Sylvius and poor filling of the cisterna pontis and interpeduncularis. A rounded mass can be seen (arrows) just behind the posterior clinoids. Normal ventriculoencephalogram is illustrated for comparison in B. (Courtesy of Dr. J. Taveras, Department of Radiology, Neurological Institute, New York.) (From: Littman, M. L. and Zimmerman, L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

hydrocephalus may result in compression atrophy of cerebral convolutions. Tumor-like granulomas may be found in any part of the brain, cord or meninges, and may be responsible for neurosurgical procedures performed in patients with cerebral cryptococcosis [13,14]. Localized cryptococcal granulomas may occur either in conjunction with diffuse leptomeningitis or without any evidence of meningitis. In addition, diffuse cryptococcal leptomeningitis in the absence of granuloma may simulate the appearance of an expanding intracranial lesion. (Fig. 8.) Although calcification of cerebral cryptococcal lesions is unusual, there is one case report of a calcified cryptococcal granuloma of the

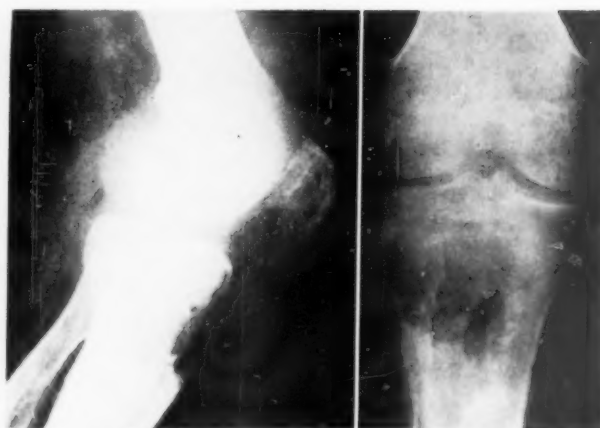


FIG. 9. Osteolytic lesion with sharply scalloped margins in proximal end of tibia. There is complete destruction of tibial tubercle. (Courtesy of Dr. R. Wigh, Department of Radiology, Presbyterian Hospital, New York City.) (From: Littman, M. L. and Zimmerman, L. E. *Cryptococcosis*. New York, 1956. Grune & Stratton.)

frontal lobe which was removed surgically. The patient remained well for one year post-operatively without any other therapy [14].

Skin and Mucous Membranes. Involvement of the skin and mucous membrane is usually a manifestation of dissemination. Approximately 10 per cent of all patients with cryptococcosis exhibit cutaneous manifestations of the disease involving face, scalp, neck, trunk and extremities [15-18]. Of the different types of skin eruptions encountered, only those of acneform characteristics appear to be of diagnostic significance. Direct extension of osseous lesions to overlying skin or subcutaneous tissue also occurs [19]. Mucous membrane lesions may appear independently or as a result of spread from a contiguous cutaneous lesion.

Bones and Joints. As with skin, bone infections represent hematogenous dissemination of the organism. Isolated bone lesions are unusual in the absence of disseminated infection. Pain and swelling usually accompany bone involvement. Although nearly every bone has been included in reported cases of cryptococcosis, joint lesions are rare and there appears to be a predilection for bony prominences, cranial bones and vertebrae [9]. (Fig. 9.) Roentgenographic examinations of bone lesions reveal that they are multiple, widely disseminated and tend to be destructive. Although chronic and slowly changing, bone lesions regress, with slow reformation of normal bone. Periosteal proliferation is not a characteristic finding.

Association with Other Diseases. The incidence of infection with *C. neoformans* is much higher

in patients with malignant disease of the reticuloendothelial system, such as Hodgkin's disease, lymphosarcoma and leukemia. Of the sixty cases of cryptococcosis studied by Zimmerman and Rappaport [20], 30 per cent of the cases appeared in patients with Hodgkin's or related diseases. In addition, cryptococcosis has been observed in association with sarcoidosis [21,22], lupus erythematosus [23], reticulum cell sarcoma [24], carcinomatosis, osteogenic sarcoma, congenital hemolytic anemia [25], tuberculous meningitis [24] and with other fungus infections. *C. neoformans* may be capable of causing disease in these patients due either to a common host susceptibility or to specific changes in the nutritional contents of their cells, plasma or tissue fluids, either of which may favor rapid growth of the organism [7].

EPIDEMIOLOGY

Cryptococcosis is world wide in distribution. The etiological agent is a pathogenic yeast which is believed to exist primarily as a saprophyte in nature. This opinion is supported by the sporadic nature of the disease, the absence of man-to-man or animal-to-man transmission, and the recovery of virulent strains of the organism from natural sources [26]. *C. neoformans* was first isolated from peaches [27]. Subsequently it was isolated from milk [28,29], soil [30,31] and pigeon excreta, first by mouse inoculation [32,33], then by direct cultural isolation [26]. The widespread distribution of the parasite is illustrated by the fact that many species of animals that have close contact with the soil, i.e., horse, cow, pig, dog, fox, cat, cheetah, civet, monkey, guinea pig and ferret, contract the disease [9].

The excreta of the pigeon (*Columba livia*) is frequently heavily infested with *C. neoformans* [26]. (Fig. 10.) The finding of large numbers of the organisms in pigeon excreta in outdoor sites in New York City and in pet shops [26] confirms the observations of previous investigators that *C. neoformans* is commonly contained in pigeon excreta [30,33]. In recent years, I have found that an increasing number of patients with primary cryptococcal meningitis have reported close contact with pigeons. In most cases, exposure of these patients to such excreta preceded the nervous system disease by several weeks. In one instance, a resident physician in New York who had worked for several hours in a hospital library near an air conditioner that was laden with pigeon excreta,



FIG. 10. Numerous colonies of *C. neoformans* (arrow) on liver-spleen glucose blood agar after plate was seeded with diluted suspension of pigeon excreta and incubated for forty-eight hours at 37°C. (From: Littman, M. L. and Schneerson, S. S. *Am. J. Hyg.*, 69: 49, 1959.)

was stricken with primary cryptococcal meningitis several weeks later. Cultures made of the pigeon excreta around the vents of the air conditioner revealed heavy infestation with a virulent strain of *C. neoformans*. Because of the known pulmonary route of infection and the heavy contamination of pigeon excreta with virulent strains of *C. neoformans*, excessive exposure to pigeon excreta dust should be avoided. The gastrointestinal route of infection also appears possible in light of the reported infections produced in monkeys [34]. Until it can be proved that infectivity of cryptococcus-laden pigeon excreta dust for human beings is low, it is advisable to institute preventive control measures for persons who have close, continued contact with pigeon excreta. This consists of disinfection of excreta dust with antiseptics, use of face masks when in proximity to pigeon coops, coop disinfection and hand disinfection [26]. It would also seem advisable for ambulatory patients with reticuloendothelial disease to avoid undue contact with pigeons and excreta dust and to shun areas where pigeons congregate.

LABORATORY DIAGNOSIS

Identification of the Organism. Cryptococcus *neoformans* is the only encapsulated yeast capa-

TABLE I
SUMMARY OF PROCEDURES UTILIZED FOR ISOLATION AND
IDENTIFICATION OF CRYPTOCOCCUS NEOFORMANS FROM
HEAVILY CONTAMINATED SPECIMENS*

| | |
|--|---|
| 1. Suspend specimen in saline, allow to settle, dilute supernatant 1 to 2 | |
| 2. Seed 2 liver-spleen glucose blood-agar plates heavily and lightly | |
| 3. Incubate 48 to 72 hours at 37°C. | |
| 4. Transfer creamy-tan, butyrous, conical yeast colonies to urea agar, corn-meal agar, Cryptococcus capsule agar, yeast nitrogen base agar containing carbohydrates and yeast carbon base agar, containing 0.078% KNO ₃ | |
| Urea agar for fungi at 20°C. | Positive hydrolysis† |
| Corn-meal agar at 20°C. | Yeast growth, no filamentation† |
| Cryptococcus capsule agar at 37°C. | All cells encapsulated (India ink mount) budding yeast cells, no filamentation† |
| Carbohydrate assimilation at 20°C. | Positive assimilation of dextrose, mannose, trehalose, xylose, galactose, maltose, sucrose, inositol, dextrin and starch; negative in lactose |
| KNO ₃ assimilation at 20°C. | Negative |
| 5. Mouse virulence | Intracerebral injection causes hydrocephalus and death in most cases within 40 days, in all cases within 73 days |

* (From: M. L. Littman and S. S. Schneierson, *Am. J. Hyg.*, 69: 49, 1959.)

† *Candida albicans* filaments and forms chlamydospores on corn-meal agar, is filamentous and non-encapsulated on Cryptococcus capsule agar and fails to hydrolyze area.

ble of invading the central nervous system of man. The demonstration of encapsulated yeast-like cells in the spinal fluid, by means of India ink* mount, therefore is prima-facie evidence of the presence of cryptococcal meningitis. Degenerated cells, oil and air droplets, other artefacts, and inexperience with the organism may lead to misdiagnosis, hence culture studies are recommended to identify the organism and confirm the clinical diagnosis. Since non-virulent saprophytic cryptococci other than *C. neoformans* are also encapsulated, the isolation

* India ink, 15 ml.; aqueous solution of merthiolate (Lilly) 1:1000, 30 ml.; tween-80 1:100 aqueous solution, 0.1 ml.; paper filtered before use.

of an encapsulated yeast from a site in the body, other than the nervous system, requires that the identity of the culture be established precisely by means of a number of biological and biochemical characteristics rather than by direct examination [26]. (Table I.) If the organism is cultivated on ordinary bacteriological peptone culture media lacking the exact nutritional requirements of the organism, only a minimal amount of capsular substance may be synthesized by the yeast, while nonetheless maintaining its virulence [35]. (Fig. 11.) India ink mounts of such cultures may show only non-encapsulated cells, the identity of which might be missed unless further culture studies are performed. The yeast can be stimulated to synthesize capsular substance by transplanting it to the synthetic culture medium, *Cryptococcus capsule agar* [1,26]. (Table I.) Following an incubation period of forty-eight hours at 37°C., an India ink mount of the agar slant growth will show an abundance of capsular substance about the yeast cells. Variation in virulence of different strains for mice limits the usefulness of virulence tests for rapid diagnosis [26]. Littman and Schneierson [26] noted that whereas seventy-three days were required for all strains to produce a lethal effect, only twenty-nine of seventy-two "pigeon" strains of *C. neoformans* caused a lethal effect in mice twenty days after single intracerebral injections of 3 million yeast cells.

Unlike saprophytic yeasts that either grow sparsely or fail entirely to grow at 37°C., *C. neoformans* grows abundantly at this temperature on most bacteriological peptone culture media that contain 1 or 2 per cent glucose. The organisms grows very rapidly on liver-spleen glucose blood agar at 37°C. [36]. *C. neoformans* is differentiated from other non-myceliated yeasts by its ability (1) to grow at 37°C., (2) to hydrolyze urea, (3) to form capsules on *Cryptococcus capsule agar*, (4) to form wide capsules in mouse brain after intracerebral injection, and (5) by its virulence for white Swiss mice [26]. The organism is differentiated from other species of cryptococci (1) by its ability to grow well at 37°C.; (2) by its ability to assimilate glucose, mannose, trehalose, xylose, galactose, maltose, sucrose, inositol, dextrin and starch, all provided as separate carbon sources in synthetic media; (3) by its failure to assimilate lactose and potassium nitrate; and (4) by its virulence for mice [26].

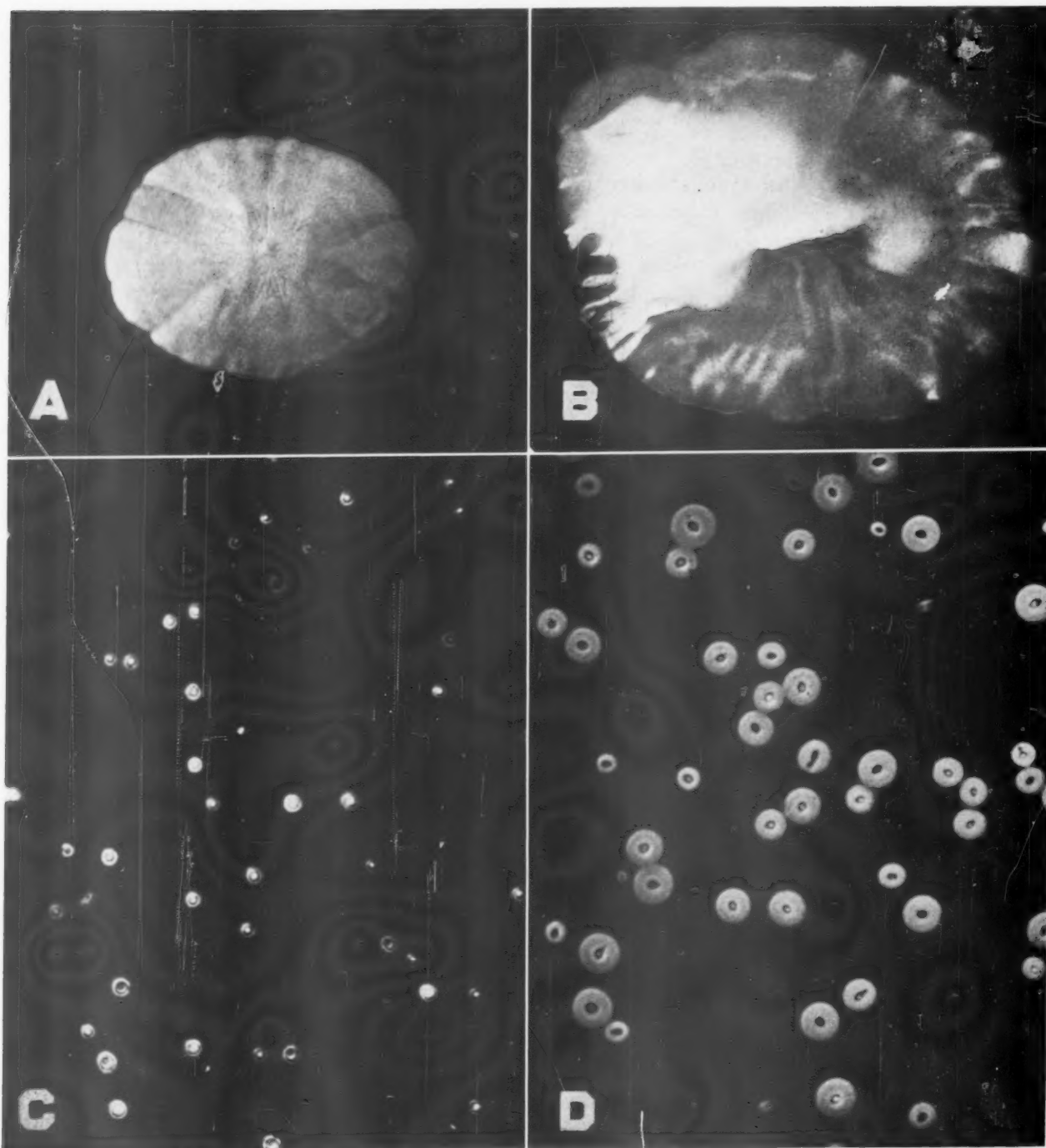


FIG. 11. Effect of culture substrate on *in vitro* encapsulation of *Cryptococcus neoformans*. A, giant colony on Sabouraud dextrose agar (SAB), sixteen days at 37°C. Original magnification, $\times 2$. B, giant colony on *Cryptococcus capsule* agar (CCA) sixteen days at 37°C. Original magnification, $\times 2$. C, India ink mount of growth from SAB colony showing absent or sparse capsules. Phase microscopy, unstained. Original magnification, $\times 300$. D, India ink mount of growth from CCA colony showing abundant capsules. Original magnification, $\times 300$. (From: Littman, M. L. and Tsubura, E. *Proc. Soc. Exper. Biol. & Med.* In press.)

C. neoformans is the only pathogenic species of the genus *Cryptococcus*. Strains of the so-called avirulent saprophytic species, *Cryptococcus neoformans* var. *innocuous* [37] were found to be either *Cryptococcus diffluens* or

Cryptococcus albidus, non-pathogenic species [38]; the species term, *innocuous*, is a misnomer, therefore, and its use should be discontinued. From all available studies, an avirulent species of *C. neoformans* does not exist.

Serological and Immunological Aspects. While recovery of *C. neoformans* from clinical and pathological material provides an unequivocal diagnosis, serological evidence of disease also can be utilized, as in histoplasmosis, to stimulate early search for the organism. This might be extremely valuable in the early diagnosis of pulmonary cryptococcosis before spread to the central nervous system has occurred. It would also be useful in the early diagnosis of cryptococcal granulomas of the brain and spinal cord and in cryptococcal meningitis when isolation of the organism from the spinal fluid either is not attempted or is negative. Serologic evidence of disease would be important in delineating the extent of primary pulmonary cryptococcosis in the population. Finally, since a fairly effective medication, amphotericin B [2-8], is now available for the treatment of cryptococcosis, early diagnosis assumes more importance than ever.

Because of improvements in microbiological and pathological techniques, cryptococcosis is now recognized far more readily than in the past. Nevertheless, the small number of cases encountered in any single medical center tends to restrict the scope of immunological studies for which a large number of positive specimens are required in the evaluation of results. For example, in two recent reports on the usefulness of complement fixation tests in the diagnosis of cryptococcosis, one research group had only two positive spinal fluids for testing and appealed for more specimens [39], while another group described results only with positive rabbit serums because none were available from human sources [40].

Another deterrent to immunological research in this disease has been the erroneous concept that *C. neoformans* is poorly antigenic. However, high-titered immune rabbit serum can be produced by injection of whole yeast cells [41,42]. Granulomatous reactions do occur in tissues, and exudation of polymorphonuclear leukocytes also occurs, although to a lesser degree. Salvin [43] recently offered the opinion that instead of being a poor antigen, *C. neoformans* produced an excess of antigen from its polysaccharide capsule during the course of infection. He considered that in the presence of an excess of polysaccharide antigen, immune antibodies might not have the opportunity to function and to manifest themselves.

C. neoformans is classified as serotypes A, B and C, by means of the agglutination test,

capsular reaction, and agglutinin absorption technic with hyperimmune rabbit serum [44]. Judging from the reaction of the organism to immune serum, type specificity resides in the capsule of the organism. The capsule of *C. neoformans* consists of two polysaccharides, an amylose which is liberated by the cell into an acid medium [45-47], and a serologically active pentosan of the hemicellulose group, not unlike gum arabic, which is liberated into a neutral medium [46-50]. Serological classification of *C. neoformans* into these three types [44,51] is based upon fine serological differences in the capsular pentosan, the acid hydrolyzed products of which have been identified by means of paper chromatographic analysis [52-54] and serologic cross reaction [55] as xylose, mannose, galactose and glucuronic acid. Although the three antigenic types of *C. neoformans* are serologically distinct, hydrolysates of their capsular polysaccharides contain the same four monosaccharides, which indicates the close antigenic relationship of the serotypes [53]. Nevertheless, cross agglutination does occur between the three serotypes, particularly between type A cells and B antiserum [56]. *C. neoformans*, moreover, is serologically related to other species of fungi [41,56,57] and to some bacteria. Polysaccharides of type A *C. neoformans*, for instance, cross react with type 14 antipneumococcal horse serum, due presumably to common galactose-end groups [55]. Cross agglutination also occurs between anti-*C. neoformans* rabbit serum and *Candida albicans*, *Saccharomyces cerevisiae* and trichophylin extract. Cross agglutination does not occur between anti-*C. neoformans* rabbit serum and *Histoplasma capsulatum*, *Blastomyces dermatitidis* or *Coccidioides immitis* [56]. Cross reaction occurs with type 2 pneumococcus, and gum tragacanth against some anticryptococcal serums. Cells of type A *C. neoformans* cross agglutinate with *Candida curvata* antiserum. Cells of *Cryptococcus diffluens*, *Cryptococcus albidus* and *Cryptococcus luteolus* cross agglutinate in both polyvalent and type A anti-*C. neoformans* serum [57].

Somatic proteins synthesized by heavily and lightly encapsulated strains of *C. neoformans* from an inorganic source of nitrogen were isolated and compared [58]. The dicarboxylic, glutamic and aspartic acids predominated among the products of acid hydrolyses of the somatic protein of both strains. Methionine was lacking in both instances. Immunological studies

of the somatic protein of the cell were not reported.

Active immunization with products of *C. neoformans* could not be accomplished in mice [59]. Untreated decapsulated cells of *C. neoformans* failed to invoke an immune response in mice, and failed to stimulate the production of agglutinins in rabbits [59]. Subcutaneous injection of whole killed cells of *C. neoformans*, decapsulated cells or crude polysaccharides failed to induce protective antibodies in mice, although purified polysaccharides, combined with resin, did protect mice to a variable degree. On the other hand, passive immunization of mice against *C. neoformans* was accomplished with immune rabbit serum [60]. Large capsule variants of *C. neoformans* were resistant to phagocytosis by mouse polymorphonuclear leukocytes, while small capsule variants were susceptible to ingestion.

Diagnostic serological procedures employing biological fluids from patients with cryptococcosis have not been studied enough to warrant conclusions regarding their present efficacy. However, the most promising technic appears to be a complement fixation test employing formalin-killed *C. neoformans* cells as antigen. In this recent complement fixation test described by Anderson and Beech [39] a single strain of *C. neoformans* of undisclosed serotype was used as the antigen. Immune rabbit serum was serially diluted in calcium-magnesium-saline solution and incubated at 37°C. for one hour with 2.5 units complement, plus the appropriate antigen or body fluid suspected of containing antigen. Red blood cells of sheep sensitized with hemolysin (amboceptor) were added and the tubes were incubated for thirty minutes at 37°C. After centrifuging, the tubes were read with 50 per cent hemolysis as end point. Positive titers of 1:40 and 1:80 were obtained with two anti-*C. neoformans* rabbit serums, and titers of 1:160 were obtained with spinal fluids from two confirmed cases of cryptococcal meningitis. Negative results in tests were obtained with numerous specimens of normal rabbit serums, normal human serums, normal urines, and spinal fluids of patients without disease of the central nervous system [39].

A somewhat different complement fixation test was described by Fischer and Labzoffsky [40] who employed formalinized cells of three strains of *C. neoformans* of undisclosed serotypes as antigen for preparation of immune rabbit

serum. Complement fixing antigen was prepared by treating yeast cells with pyridine, then washing the cells with saline solution and subjecting them to the disruptive effect of ultrasonic vibration.

Salvin [47] compared complement fixation and agglutination technics for the detection of anti-*C. neoformans* antibodies in immune rabbit serums, and demonstrated that the former technic was by far the more sensitive. In view of the serologic heterogeneity of *C. neoformans*, the use of antigen of all three serotypes with polyvalent rather than monovalent immune serums would increase the range of the complement fixation test.

A different approach to the serologic diagnosis of cryptococcosis was described by Neill, Sugg and McCauley [61] who employed spinal fluid, blood and urine from a patient with cryptococcosis as antigen rather than as antibody. Positive precipitin and complement fixation tests were obtained with all three fluids. The demonstration of a soluble antigen in the patient's serum in the absence of a positive blood culture for *C. neoformans* illustrates the importance of serologic testing. Absorption of immune serum with cryptococcal polysaccharide removed its capacity to react further with the patient's spinal fluid and blood serum, indicating that the immunologic reactions were specific and were concerned with capsular polysaccharides. Salvin [43] points out that these data lend support to the view that the host may have an excess of antigen and hence difficulty in metabolizing the capsular polysaccharides of *C. neoformans*, thus permitting the polysaccharides to remain in the cells and tissues for long periods of time. The capsular reaction of *C. neoformans* with immune serum is similar in principle to the Neufeld "quellung" test for pneumococci [48,62]. Cells washed in saline solution and combined with immune serum and a small amount of methylene blue give a capsular reaction in the presence of homologous but not of heterologous serum. However, cross reactions may occur with saprophytic cryptococci. *C. diffluens* and *C. luteolus* give capsular reactions in polyvalent and type A *C. neoformans* antiserum, while *C. albidus* reacts with polyvalent and types A and B antiserum [57]. The capsular reactivity of the immune serum is related to its agglutinin and precipitin titers, since absorption of immune serum with homologous antigen will remove the capsular reactivity of the serum.

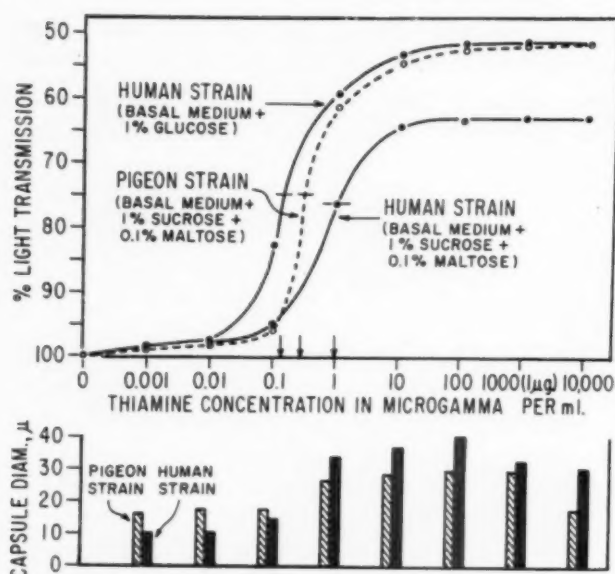


FIG. 12. Effect of thiamin on growth and capsule synthesis of vitamin-depleted strains of *C. neoformans*, in vitamin-free basal salt medium measured after thirty days' incubation at 37°C., quiescent culture. Half-maximal value of thiamin is 0.2 $\mu\text{g./ml.}$ (0.0002 $\mu\text{g./ml.}$) (From: Littman, M. L. *Tr. New York Acad. Sc., Series II*, 20: 623, 1958.)

The capsule does not swell in the presence of antibody. Exposure of washed encapsulated cells of *C. neoformans* to specific antibodies causes prominent clarification of capsular outline without an increase in capsule size, as determined by packed cell volume and direct microscopic measurement [63].

Relatively little is known about the allergic state in cryptococcosis and there is still no satisfactory antigen for eliciting skin hypersensitivity. In rabbits immunized with *C. neoformans* non-specific erythema and induration of the skin develop after intradermal injections of high concentrations of capsular substance, but not when more diluted concentrations are injected [64]. Positive skin reactions have been recorded in patients after intradermal or subcutaneous injections of boiled aqueous extract of *C. neoformans* [65], broth filtrate [66], saline suspension [67], heat-treated saline suspension, acid-treated saline suspension [68], and similar materials [69]. In most of these instances, however, crude concentrated material was injected, so that the skin reactions may represent non-specific reactions. When pure polysaccharides of serotypes A, B and C are injected intradermally into patients with confirmed cryptococcosis, positive skin reactions are not elicited [23].

Prevalence. Because of the widespread nature of the parasite in the soil [31,70] and in the environment [9,26,28-33], a large segment of our population undoubtedly is being exposed to the fungus. Judging from the emergence of a single case of fungal meningitis from 2,000 subclinical and clinical cases of mycoses, such as coccidioidomycosis [71], it has been postulated that 5,000 to 15,000 cases of subclinical or clinical pulmonary cryptococcosis exist every year in New York City alone, if the same ratio is applied for cryptococcosis [26]. Since the disease is sporadic in nature, research programs, particularly immunological, would be greatly aided by centralization of cases for qualified studies.

Biochemical Aspects of *C. Neoformans*. Studies of the characteristics of *C. neoformans* reveal certain *in vivo* and *in vitro* phenomena. One is the predilection of *C. neoformans* for the central nervous system, particularly the spinal fluid and meninges. Another is the loss of capsular substance by the organism when transferred from an *in vivo* site to an artificial culture medium. Still another is the rapid synthesis *in vivo* of large capsules by ordinarily weakly-encapsulated laboratory strains, when injected intracerebrally into mice. Contrary to common belief, continued maintenance of *C. neoformans* on carbohydrate-rich culture media does not restore the capsule to *in vivo* size. It is as though *C. neoformans* finds some essential metabolites or optimal conditions in the brain and spinal fluid that furnish the stimulus for growth and synthesis of large capsules [7].

Recent studies have pointed out the vitamin, amino acid and carbohydrate requirements of *C. neoformans* and their influence upon capsule synthesis [7]. It was found that *C. neoformans* was thiamin-deficient in that the organism possessed an absolute requirement for thiamin and its moieties, and showed a complete lack of response to all other water soluble vitamins. Concentrations of thiamin ranging from 0.00001 to 0.1 $\mu\text{g./ml.}$, when added to a glucose synthetic basal medium, stimulated the synthesis of capsular substance by *C. neoformans*. (Fig. 12.) The organism was capable of separately synthesizing the pyrimidine and thiazole components of thiamins, and of combining them into thiamin. The half-maximal growth value of thiamin for *C. neoformans* proved to be 0.0002 $\mu\text{g./ml.}$ [7]. In comparison the thiamin content of normal and abnormal spinal fluid varies from 0 to 0.0150 $\mu\text{g./ml.}$ [72,73]. Since the thiamin

level of spinal fluid is sharply increased by intravenous or intramuscular injection of thiamin [72,73], and since *C. neoformans* is wholly dependent upon this vitamin for the synthesis of its capsular substance, it has been advised that thiamin or vitamin B complex be withheld from patients suffering from cryptococcal meningitis [7]. In this connection it is of interest that large capsule variants of *C. neoformans* resist phagocytosis by mouse polymorphonuclear leukocytes, whereas small capsule variants are susceptible [60].

In order for *C. neoformans* to synthesize its capsular polysaccharides from a single nutrient in the central nervous system and, from the same source synthesize its somatic proteins, carbohydrates, fats and enzymes, it would be necessary for that nutrient to contain the precursors necessary for the biosynthetic pathways of nitrogen, carbohydrate and fat metabolism [7]. A compound satisfying many of these requirements is glutamic acid, which participates in reactions of transamination and is therefore involved in the biological synthesis of amino acids. It undergoes reversible oxidative deamination to α -ketoglutaric acid, an important intermediate in the tricarboxylic acid cycle, and is also capable of decarboxylation to γ -aminobutyric acid. Carbon-depleted cells of *C. neoformans* in thiamin-ammonium sulfate basal salt medium utilize L-glutamine, DL-, L- and D-forms of glutamic acid, and sodium glutamate as sole carbon sources, and produce wide capsules as well as intracellular inclusions of neutral fat [7]. (Fig. 13.) Growth-stimulatory effect of sodium glutamate is observed up to 2 mg./ml. while both growth-inhibiting and capsule-inhibiting effects appear in concentrations exceeding 10 mg./ml. [7]. When thirty-one amino acids and derivatives are furnished to *C. neoformans* as sole separate sources of carbon, the organism readily assimilates glutamic acid, L-proline, with minor assimilation of DL-serine and L-asparagine, but fails to assimilate any other amino acid source [7]. It is of interest to note that all anatomic divisions of the brain contain a considerable quantity of free L-glutamic acid (2.51 to 3.45 mg./ml. of moist tissue), while the adult spinal cord has an average content of 4.75 mg./ml. moist tissue [74]. Coincidentally, these are also optimal concentrations for *C. neoformans*. (Fig. 13.) Since proline is absent from normal spinal fluid, glutamic acid and glutamine form the major sources of amino com-

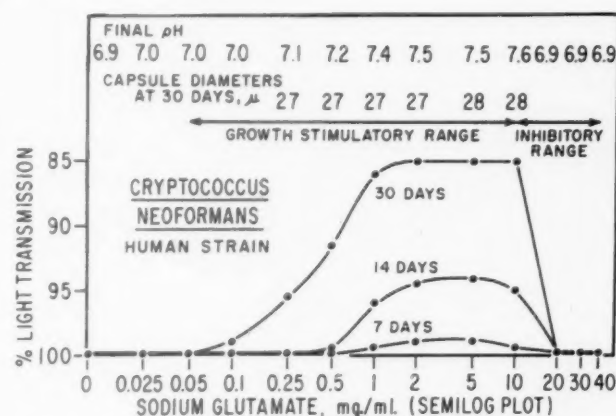


FIG. 13. Utilization of sodium glutamate as sole source of carbon by *C. neoformans* (human strain) in thiamin-basal salt medium, incubated at 37°C., quiescent culture. (From: Littman, M. L. *Tr. New York Acad. Sc.*, Series II, 20: 623, 1958.)

pounds in the spinal fluid that are available to the organism as readily assimilable but secondary sources of carbon [7].

Although *C. neoformans* is not considered a fermentative yeast, it readily assimilates all carbohydrates and derivatives present in normal spinal fluid, such as glucose, fructose, glucosamine, mannose, galactose, inositol, α -ketoglutaric acid and glucuronic acid, when these are furnished as sole separate sources of carbon in a synthetic medium [7]. (Table II.) The organism has assimilative preference for unbranched polysaccharides and disaccharides containing α -glucoside structures and fails to assimilate branched polysaccharides such as glycogen. *C. neoformans* is capable of synthesizing neutral fat from all carbohydrate sources that it is capable of assimilating.

Energy-Yielding Metabolic Pathways. Carbohydrate substrates serve as major sources of energy for biosynthesis by *C. neoformans*, the amino acids, glutamic acid and proline serving as secondary sources [7]. Glutamic acid, L-proline and α -ketoglutaric acid form important links through which the oxidative breakdown of protein is connected with that of carbohydrates. Evidence for functioning of the Krebs's tricarboxylic acid cycle [75,76] is found in the ability of the organism to utilize α -ketoglutaric acid, and to assimilate aldohexoses, ketohexoses and glyceraldehyde readily [7]. The organism fails to accept intermediates of the Embden-Meyerhof glycolytic system or monophosphate shunt [7].

Energy-Consuming Biosynthetic Pathways. The starting point for synthesis of protein by *C. neo-*

TABLE II
COMPOSITION OF NORMAL HUMAN CEREBROSPINAL FLUID *

| Component | μg./ml. |
|---|---------|
| Amino acids | |
| Aspartic acid..... | 1.5 |
| Alanine..... | 1.8 |
| Arginine..... | 1.2 |
| α-Aminobutyric acid..... | 0.2 |
| Cystine..... | 0† |
| Glycine..... | 1.4 |
| Glutamic acid..... | 1.4 |
| Glutamine..... | 3.0 |
| γ-Aminobutyric acid..... | 0.2 |
| Histidine..... | 0† |
| Lysine..... | 1.9 |
| Leucine..... | 1.2 |
| Phenylalanine..... | 1.8 |
| Proline..... | 0† |
| Serine..... | 2.5 |
| Tyrosine..... | 0.8 |
| Threonine..... | 1.4 |
| Valine..... | 1.0 |
| Proteins and nitrogenous compounds | |
| Albumin..... | 550 |
| Fibrinogen..... | 0 |
| α-Globulin..... | 100 |
| β-Globulin..... | 120 |
| γ-Globulin..... | 110 |
| Non-protein N..... | 190 |
| Creatinine..... | 12 |
| Urea..... | 117 |
| Carbohydrates | |
| Reducing substances‡ (including glucose) | 670 |
| Fructose..... | 38 |
| Glucosamine..... | 11 |
| Glucuronic acid..... | Present |
| Inositol..... | 29 |
| Mannose..... | Present |
| Galactose..... | Present |
| Pentose, unidentified..... | Present |
| Fucose..... | Present |
| α-Ketoglutaric acid..... | 0.7 |
| α-Oxoglutaric acid..... | 0.2 |
| Pyruvic acid..... | 1.7 |
| Ascorbic acid..... | 6 |
| Citric acid..... | 0.4 |
| Lactic acid..... | 170 |
| Elements | |
| Al, Ba, B, Ca, Cu, I, Mg, P, K, Na, Sr, S, Fe, HCO ₃ ⁻ , Cl ⁻ | Present |
| Physical properties | |
| Specific gravity..... | 1.007 |
| pH..... | 7.4 |
| CO ₂ vol. %..... | 59 |

* (From: M. L. Littman, *Tr. New York Acad. Sc.*, Series II, 20: 623, 1958.)

† Present in abnormal spinal fluids.

‡ Twenty-five per cent is non-sugar.

formans appears to be ammonia as the primary source of nitrogen, in the presence of carbohydrates or amino acids to supply carbon and energy. Glutamic acid, proline, asparagine and α-ketoglutaric acid are assimilated by *C. neoformans* as sole sources of carbon and energy. The most likely pathway, therefore, for conversion of ammonia to α-amino acids is considered to be by way of ketoglutarate, through the hydrogenation of an α-ketodicarboxy acid to L-glutamic acid. The presence of glutamic oxalacetic transaminase in cryptococcal cell-free filtrates and the utilization of glutamic acid and α-ketoglutaric acid indicate that *C. neoformans* is capable of biosynthesis of its natural amino acids by transamination [7]. Uzman et al. [58] have shown that *C. neoformans* is capable of the synthesis of sixteen amino acids from ammonium sulfate and glucose when grown in a thiamin-mineral synthetic medium.

Starting points for carbohydrates of the cryptococcal cell and capsule may be any one of a wide variety of simple or complex carbohydrates varying from glyceraldehyde to starch, for all of which the organism apparently possesses the necessary enzyme systems. The carbohydrate building block appears to be a simple three-carbon sugar, such as glyceraldehyde, which is readily assimilated, and from which the organism is able to synthesize its intracellular carbohydrates and capsular polysaccharides [7].

Lipids of *C. neoformans* appear in the cell as small, intracellular inclusions. Judging from its absorption of Sudan iv, the lipid inclusions are composed primarily of neutral fat. Starting points for lipid synthesis by *C. neoformans* apparently are furnished by a wide variety of carbohydrates, as well as by the amino acids, glutamic acid, L-proline, L-asparagine, and DL-serine. *C. neoformans* does not utilize lecithin or the fatty acids, oleic and stearic acids as sole sources of carbon, and it assimilates glycerol poorly. The pathway of lipid synthesis for *C. neoformans* is unknown at present [7].

The nuclear chromatin of *C. neoformans*, deoxyribonucleic acid (DNA), is synthesized by the organism from the carbohydrates, D-ribose, D-glucose, sucrose and soluble starch [7].

Infection of the central nervous system of man with *C. neoformans* is an example of the exquisitely selective parasitism that occurs when the exact nutritional needs of a parasite are supplied by a particular host tissue. The unusual

affinity of *C. neoformans* for the central nervous system is considered to be due to the presence of optimal quantities of thiamin, glutamic acid and glutamine, and a variety of assimilable spinal fluid carbohydrates and minerals. The disseminated nature of cryptococcosis in reticulo-endothelial disease is thought to be due in part to specific change in blood cells and plasma [1] and to some host susceptibility that favors the growth of *C. neoformans*.

TREATMENT

Because of the high mortality in cryptococcal meningitis and disseminated cryptococcosis, and the absence of specific therapy, the disease has been treated empirically with a wide variety of agents, including fever therapy, ultraviolet radiation, x-rays, heavy metals, sulfonamides, diamidines, antibiotics, immunologic agents, enzymes and others [9]. Several hundred miscellaneous chemical compounds have been screened for *in vitro* effectiveness against *C. neoformans* [77,78]. Since spontaneous remissions occur in some untreated cases [9,79], credit for a curative effect has often been erroneously ascribed to medications subsequently proved to be useless on further clinical trial. This reaffirms the necessity for long term observation in evaluating any therapy for cryptococcosis.

The first truly antifungal antibiotic to be applied to the treatment of cryptococcal meningitis was cycloheximide (Actidione®), an antibiotic that possesses high *in vitro* fungicidal activity against *C. neoformans* and other yeasts [80,81]. However, following a series of clinical trials of Actidione by more than a score of investigative groups, it became obvious that this antibiotic was clinically ineffective [9]. Shortly after the introduction of the diamidines and their successful use in the therapy of North American blastomycosis [82,83], these also were applied in an attempt to cure cryptococcosis [84-86]. However, an unsuccessful trial of stilbamidine, administered intravenously to three cases of cryptococcal meningitis, was reported by Miller and associates [87]. The failure of the drug to affect the clinical course of the disease was consistent with the relative resistance of *C. neoformans in vitro*, as compared with the more sensitive *Blastomyces dermatitidis* [88,89].

Surgical treatment without antibiotic therapy has been successful in eradicating isolated foci of infection. Spread of cryptococcal infection from the lungs to other tissues is not always ac-

companied by diffuse meningoencephalitis or widespread dissemination. In these cases, surgical excision of extrapulmonary sites of localization has successfully eradicated the foci of infection [90,94].

Amphotericin B. In the continued search for clinically effective antifungal agents, attention is currently focused on amphotericin B,* a polyene, antifungal antibiotic isolated in 1955 by Gold, Stout, Pagano and Donovan [95]. A review of the physical, chemical and biological properties of amphotericin B, its *in vitro* spectrum, mode of action, and pharmacological actions in animals and man appeared elsewhere [96,97]. The consensus of investigators is that amphotericin B, when used intravenously, is highly effective in the treatment of disseminated mycoses, such as North American blastomycosis, South American blastomycosis, histoplasmosis, coccidioidomycosis, cryptococcosis and moniliasis [97]. No permanent adverse effects upon heart, liver, bone marrow, central nervous system or skin have been observed. Azotemia occurs when the dose is excessive or too frequent, in which case administration must be limited to alternate days. Side effects of the antibiotic include nausea, occasional vomiting, flushing, perspiration, fatigue, drowsiness, febrile reactions, anxiety and generalized pain, all of which usually subside upon discontinuation of the medication. Thrombophlebitis may occur but its incidence is low, particularly if 2 to 4 mg. of heparin is added to the infusion solution. Maximum serum levels in patients receiving amphotericin B intravenously in dosage of 1.2 mg./kg./day are usually no higher than 1.8 µg./ml. [96]. This exceeds the minimal inhibitory concentration required for most of the systemic pathogenic fungi, including *C. neoformans*. Since adequate blood serum levels of the antibiotic are still present twenty-four hours after an infusion, therapy on alternate days is routinely employed to avoid the azotemia that usually accompanies daily treatment. The persistence of amphotericin B serum levels at twelve, eighteen and twenty-four hours after a single infusion of the antibiotic is ascribed to its high renal threshold, associated with a prolonged biological half-life [96]. The minimal inhibitory concentration of amphotericin B for *C. neoformans* ranges from 0.2 to 0.3 µg./ml. in glucose Penassay broth incubated

* The trade name of E. R. Squibb and Sons, Division of Olin Mathieson Chemical Corporation, for amphotericin B is Fungizone.®

for forty-eight hours. This antibiotic is both fungistatic and fungicidal for the systemic fungi, including *C. neoformans* [96,98].

Patients with leukemia and with complications of extraneural monilial or cryptococcal dissemination may be cleared of the fungal parasites with a relatively small number of infusions. This is due to the susceptibility of the organisms to amphotericin B and to the presence of sustained fungicidal levels in blood serum and tissue fluids produced by intravenous administration. In the case of fungi more resistant to the action of amphotericin B, such as those causing coccidioidomycosis, histoplasmosis and North American blastomycosis, therapy is prolonged for several months until a minimum of 2,000 to 3,000 mg. is administered. The bone marrow remains normal during prolonged therapy with amphotericin B but normocytic hypochromic anemia appears in some patients, and may require the administration of one or two blood transfusions.

One of the problems associated with the use of amphotericin B in the treatment of fungal meningitides is the failure of the antibiotic to cross the blood-brain barrier in sufficient concentration to effect rapid eradication of the fungus from the central nervous system [97]. Despite the presence of adequate antibiotic levels in the blood stream following intravenous administration, the spinal fluid fails to contain any measurable levels [5,6,96]. Since the method of bioassay with a standard strain of *C. albicans* [96] will not detect levels below 0.18 $\mu\text{g./ml.}$, it may very well be that amphotericin B levels in the spinal fluid are below this point. With the use of a more sensitive assay technic [7], a level of amphotericin B of 0.09 $\mu\text{g./ml.}$ has been reported in spinal fluid following intravenous administration. Despite the low level of amphotericin B in the spinal fluid, however, many patients with cryptococcal meningitis treated intravenously with amphotericin B show a favorable clinical response [2-8,99]. The low level of antibiotic in the spinal fluid is apparently sufficient to inhibit more sensitive strains of *C. neoformans*. In a smaller group of patients with cryptococcal meningitis, presumably infected with more resistant strains, the spinal fluid remains consistently positive for *C. neoformans*, despite prolonged intravenous therapy with amphotericin B [99], and relapses occur. It is necessary, therefore, to treat these patients by intrathecal as well as intravenous injection.

When a single dose of 0.5 mg. amphotericin B dissolved in distilled water or patient's spinal fluid is injected intrathecally, it promptly produces a fungicidal level of 0.45 $\mu\text{g./ml.}$ in the spinal fluid which persists for approximately twenty-four hours, disappearing, however, within forty-eight hours [96]. Intrathecal injections not exceeding 0.7 mg. per single dose for an adult are administered on alternate days concomitant with intravenous administration until the spinal fluid is sterile. The margin between therapeutic and toxic effect in intrathecal injection is narrow, since a single dose of 1 mg. amphotericin B, which is considered an overdose, may cause hyperpyrexia, arachnoiditis, weakness of the lower extremities, flaccid paraplegia and cord bladder. These complications are reversible, however, when the drug is discontinued. Since amphotericin B possesses no antibacterial activity, intrathecal administration is performed under careful asepsis to avoid bacterial meningitis. Routine spinal fluid cultures for the presence of bacteria are necessary during the course of intrathecal amphotericin B therapy.

OTHER THERAPEUTIC MEASURES

Effect of Thiamin. In view of the importance of thiamin for the capsule synthesis of *C. neoformans* [7] (Fig. 12), and since the thiamin level in the spinal fluid is increased sharply by intravenous or intramuscular injection of the vitamin [72,73], attempts should be made to reduce the thiamin content of the spinal fluid during therapy with amphotericin B. Injections of thiamin or thiamin-rich vitamin B complex are now avoided. Vitamins of the B complex group other than thiamin may be administered separately, however. Patients with cryptococcal meningitis have been maintained on a low thiamin diet for periods of as long as three months during treatment with amphotericin B, without symptoms of polyneuritis or beri-beri developing [100].

Effect of Gamma Globulin. The failure of patients to resist bacterial infection has been attributed by Bruton [101] to the defective synthesis of gamma globulin. Examples of this are congenital agammaglobulinemia and acquired agammaglobulinemia of the idiopathic variety, as well as a type associated with granulomatous or neoplastic disease of lymphoid tissue [102]. In persons capable of immune responses, however, both gamma and α_1 human serum globulins increase sharply with

TABLE III
CLINICAL RESULTS WITH AMPHOTERICIN B IN ELEVEN CASES OF CRYPTOCOCCAL MENINGITIS AND IN ONE CASE OF PULMONARY CRYPTOCOCCOSIS

| Pretreatment | | | | | Treatment | | | | Posttreatment | | |
|-------------------------------|---|---------------------------------------|---|----------------------------------|------------------------------------|--|------------------------|--|--------------------|--|----------------------------|
| Age (yr.), Race and Sex | Clinical Status | Duration | Associated Diagnosis | Serum γ -glob- ulin | Amphotericin B Therapy | | Low Thiamin Diet | Intra- muscular γ -glob- ulin, (0.45 cc./ kg./wk.) | Follow Up | Results | Spinal Fluid Culture |
| | | | | | Total Intra- venous (mg.) | No. of 0.7 mg. Doses Intra- thecally | | | | | |
| 21, W, F | Fair, menin- gitis, head- ache | Headache, 3 wk. Cough, 3 mo. | Pregnant (2nd trimester) | | 2000 | 0 | No | No | 6 mo. | Normal ac- tivity, suc- cessful pregnancy | Negative |
| 39, W, M | Poor, menin- gitis | 6 yr. | | | 1350 | 9 | No | No | 24 mo. | Normal ac- tivity | Negative |
| 41, W, M | Poor, menin- gitis | 6 mo. | | Low | 1800 | 19 | No | No | | Died | Positive |
| 30, W, M | Meningitis | 1 mo. | Hodgkin's rheumatic heart disease (Meticorten® 2 yr.) | | 500 | 2 | No | No | | Died | Negative |
| 60, W, M | Poor, menin- gitis | 12 mo. | Hodgkin's dis- ease | | 300 | 3 | No | No | | Died | Positive |
| 57, C, F | Fair, menin- gitis, ataxia | 2½ mo. | | Normal | 1500 | 12 | Yes | No | 12 mo. | Normal ac- tivity | Negative |
| 60, W, F | Poor, menin- gitis | 3 mo. | Hodgkin's dis- ease | | 1400 | None | Yes | No | Under treatment | Considerably improved | Negative |
| 31, W, M | Fair, menin- gitis | 1 mo. | | Low | 2500 | None | Yes | Yes | 6 mo. | Normal ac- tivity | Negative |
| 55, W, F | Fair, menin- gitis | 2 mo. | Hemolytic anemia | Normal | 2000 | None | Yes | Yes | 3 mo. | Normal ac- tivity | Negative |
| 74, W, F | Poor, menin- gitis | 3 mo. | Macroglob- ulinemia | Low | 1000 | 8 | Yes | Yes | Under treatment | Considerably improved | Negative |
| 37, W, M | Poor, menin- gitis, cere- bral cyst | 6 mo. | | Low | 900 | 5 | Yes | Yes | | Died | Negative |
| 34, W, M | Pulmonary cryptococ- cosis | 4 mo. | | | 2000 | None | No | No | 12 mo. | Normal ac- tivity | Negative |

acute febrile disease [103-107]. It is believed that the failure of this immunologic response to occur in certain persons may account for the inability to cope with *C. neoformans* [9]. Gamma globulin is of value in supplementing antibiotics in the control of experimental infections in animals [108-111] and in man [111], and in infections refractory to antibiotics [112-114]. The administration of hyperimmune gamma globulin has been effective in preventing pertussis in about 75 per cent of non-immune children exposed to the disease [115]. Gamma globulin has been administered to mice with therapeutic effect, for infections due to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Streptococcus pyogenes* and *Diplococcus pneumoniae* [116]. The exact mechanism of the action of gamma globulin is not yet clear, but present evidence indicates that protection is

provided by its specific antibody content rather than by non-specific factors [117]. Since gamma globulin and various antibiotics act synergistically against a number of bacterial species [108], combined therapy has been successful in the treatment of chronic osteomyelitis and chronic pyelonephritis, after antibiotics had proved ineffective [117].

In patients who receive prolonged treatment with steroids and broad-spectrum antibiotics, localized and systemic moniliasis and other fungus infections are more prone to develop. Coincidentally, synthesis of specific gamma globulin has been depressed experimentally with ACTH, cortisone [118] and hydrocortisone [119], when administered to certain laboratory animals in large doses before and after the administration of antigen. The effects of steroids appear to vary from one species to another [120], however,

and they fail to influence the catabolism of gamma globulin [127], consequently their ability to depress antibody formation has been questioned [102]. Roentgen rays and nitrogen mustards also inhibit antibody synthesis [122], resulting in involution of lymphoid tissues. Since disseminated cryptococcosis is especially prone to develop in patients with malignant disease of lymphoid tissue who are treated with roentgen ray and/or nitrogen mustards, a prime cause of the fungus disease may be the development of hypogammaglobulinemia.

In order to explore this possibility, serum and spinal fluid electrophoresis studies were undertaken in patients with cryptococcal meningitis. In my study of eleven patients with this disease serum electrophoresis studies were performed in six cases. (Table III.) Four patients showed either markedly decreased or low normal gamma globulin values, as measured by either electrophoresis or the zinc turbidity test; two patients had normal levels. This incidence is far too great to be coincidental. When gamma globulin was administered intramuscularly twice weekly in divided doses of 0.45 cc./kg. to patients with cryptococcal meningitis, in addition to intravenous and intrathecal administration of amphotericin B and low thiamin diet, the clinical responses were excellent. The role of gamma globulin is difficult to assess in these few cases, however, since a combination of therapeutic agents was given. Of four patients who received gamma globulin in addition to amphotericin B, three recovered and one died before a full course of amphotericin B had been given. Of the eleven patients with cryptococcal meningitis, who received amphotericin B intravenously, seven improved considerably or returned to normal activity, while four patients died. (Table III.) Of these four fatal cases three patients had received a minimal number of infusions of amphotericin B before death and only one received gamma globulin concomitantly with the antibiotic. In these cases it is likely that specific antifungal therapy with amphotericin B had been started too late in the course of the disease.

SUMMARY

Epidemiological studies emphasize the wide distribution of *Cryptococcus neoformans* in nature. The disease process begins with inhalation of infected dust. There are wide variations in chest roentgenograms in pulmonary cryptococ-

cosis making it necessary to include the disease in the differential diagnosis of pulmonary diseases of slow evolution. Cryptococcal involvement of the skin, mucous membranes, bones and joints usually represents hematogenous dissemination of the organism. The clinical symptomatology of cryptococcal meningitis and meningoencephalitis is dependent upon whether the cranial infection is diffuse or localized. Pathological changes in the nervous system are those of meningitis, meningoencephalitis or of demarcated granulomas.

The diagnosis of cryptococcosis is made by isolation of the organism and confirmed by an identification procedure that utilizes a number of biological and biochemical characteristics of the organism. *C. neoformans* is classified into three serotypes on the basis of type specific capsular polysaccharide, although cross reactions between these types and other microorganisms occur. Diagnostic serological procedures, including complement fixation tests, may provide early evidence of the disease. There is still no satisfactory antigen for eliciting skin hypersensitivity.

Biochemical studies of the organism reveal its requirement for thiamin and thiamin-moieties, the need for glutamic acid and glutamine, and its ability to assimilate readily carbohydrates and carbohydrate derivatives that are present in normal spinal fluid. Carbohydrate substrates serve as major sources of energy for biosynthesis. Infection of the central nervous system by *C. neoformans* is considered to be an example of exquisite parasitism that occurs when the exact nutritional needs of the parasite are supplied by host tissue.

Eleven patients with cryptococcal meningitis were treated with amphotericin B by intravenous and intrathecal routes. Seven of these improved considerably or returned to normal activity; four patients died. Of the four fatal cases, three patients had received only a minimal number of infusions of the antibiotic before death. The clinical responses were excellent in four patients with cryptococcal meningitis who received hyperimmune gamma globulin twice weekly, intravenous and intrathecal amphotericin B, and who were maintained on low thiamin diets.

REFERENCES

1. LITTMAN, M. L. Capsule synthesis by *Cryptococcus neoformans*. *Tr. New York Acad. Sc.*, Series II, 20: 623, 1958.

2. APPELBAUM, E. and SHTOKALCO, S. Cryptococcus meningitis arrested with amphotericin B. *Ann. Int. Med.*, 47: 346, 1957.
3. RUBIN, H., LEHAN, P. H., FITZPATRICK, M. J. and FURCOLOW, M. L. Amphotericin B in the treatment of cryptococcal meningitis, p. 71. In: *Antibiotics Annual 1957-1958*. New York, 1958. Medical Encyclopedia, Inc.
4. UTZ, J. P., LOURIA, D. B., FEDER, N., EMMONS, C. W. and McCULLOUGH, N. B. A report of clinical studies on the use of amphotericin B in patients with systemic fungal diseases, p. 65. In: *Antibiotics Annual 1957-1958*. New York, 1958. Medical Encyclopedia, Inc.
5. FITZPATRICK, M. J., RUBIN, H. and POSER, C. M. The treatment of cryptococcal meningitis with amphotericin B, a new fungicidal agent. *Ann. Int. Med.*, 49: 249, 1958.
6. RUBIN, H. and FURCOLOW, M. L. Promising results in cryptococcal meningitis. *Neurology*, 8: 590, 1958.
7. McCULLOUGH, N. B., LOURIA, D. B., HILBISH, T. F., THOMAS, L. B. and EMMONS, C. Cryptococcosis: clinical Staff Conference at the National Institutes of Health. *Ann. Int. Med.*, 49: 642, 1958.
8. UTZ, J. P., TREGER, A., McCULLOUGH, N. B. and EMMONS, C. W. Amphotericin B: intravenous use in 21 patients with systemic fungal diseases, p. 628. In: *Antibiotics Annual 1958-1959*. New York, 1959. Medical Encyclopedia, Inc.
9. LITTMAN, M. L. and ZIMMERMAN, L. E. Cryptococcosis, Torulosis or European Blastomycosis, pp. 1-205. New York, 1956. Grune & Stratton.
10. RATCLIFFE, H. E. and COOK, W. R. Cryptococcosis. Review of the literature and report of a case with initial pulmonary findings. *U. S. Armed Forces M. J.*, 1: 957, 1950.
11. WOLFE, J. N. and JACOBSON, G. Roentgen manifestations of torulosis (cryptococcosis). *Am. J. Roentgenol.*, 79: 216, 1958.
12. BAKER, R. D. and HAUGEN, R. K. Tissue changes and tissue diagnosis in cryptococcosis. A study of 26 cases. *Am. J. Clin. Path.*, 25: 14, 1955.
13. CARTON, C. A. and MOUNT, L. A. Neurosurgical aspects of cryptococcosis. *J. Neurosurg.*, 8: 143, 1951.
14. LIU, C. T. Intracerebral cryptococcal granuloma. *J. Neurosurg.*, 10: 686, 1953.
15. CAWLEY, E. P., GREKIN, R. H. and CURTIS, A. C. Torulosis. A review of the cutaneous and adjoining mucous membrane manifestations. *J. Invest. Dermat.*, 14: 327, 1950.
16. LINELL, F., MAGNUSSON, B. and NORDÉN, Å. Cryptococcus (torulos), ett fall med symptom från hud, lungor och centrala nervsystemet. *Nord. med.*, 46: 1195, 1951.
17. MOORE, M. Cryptococcosis with cutaneous manifestations. Four cases with a review of published reports. *J. Invest. Dermat.*, 28: 159, 1957.
18. CROUNSE, R. G. and LERNER, A. B. Cryptococcosis. Case with unusual skin lesions and favorable response to amphotericin B therapy. *Arch. Dermat. & Syph.*, 77: 210, 1958.
19. NIÑO F. L. Contribución al estudio de las blastomycosis en la república argentina. v. Granuloma criptococcico. Estudio de un nuevo caso argentino. *Bol. d. Inst. clín. quir.*, 14: 656, 1938.
20. ZIMMERMAN, L. E. and RAPPAPORT, H. Occurrence of cryptococcosis in patients with malignant disease of the reticuloendothelial system. *Am. J. Clin. Path.*, 24: 1050, 1954.
21. COLLINS, V. P., GELLHORN, A. and TRIMBLE, J. R. The coincidence of cryptococcosis and disease of the reticulo-endothelial and lymphatic systems. *Cancer*, 4: 883, 1951.
22. PLUMMER, N. S., SYMMERS, W. St. C. and WINNER, H. I. Sarcoidosis in identical twins with torulosis as a complication in one case. *Brit. M. J.*, 2: 599, 1957.
23. LITTMAN, M. L. (Unpublished results.)
24. BURROWS, B. and BARCLAY, W. R. Combined cryptococcal and tuberculous meningitis complicating reticulum cell sarcoma. *Am. Rev. Tuberc.*, 78: 760, 1958.
25. ATKINSON, J. B., DELANEY, W. E. and MILLER, F. R. Cryptococcus meningitis in a case of congenital hemolytic anemia. *Ann. Int. Med.*, 44: 1015, 1956.
26. LITTMAN, M. L. and SCHNEIERSON, S. S. Cryptococcus neoformans in pigeon excreta in New York City. *Am. J. Hyg.*, 69: 49, 1959.
27. SANFELICE, F. Contributo alla morfologia e biologia dei blastomiceti che si sviluppano nei succhi de alcuni frutti. *Ann. d'ig.*, 4: 463, 1894.
28. KLEIN, E. Pathogenic microbes in milk. *J. Hyg.*, 1: 78, 1901.
29. CARTER, H. S. and YOUNG, J. L. Note on the isolation of Cryptococcus neoformans from a sample of milk. *J. Path. & Bact.*, 62: 271, 1950.
30. EMMONS, C. W. The natural occurrence in animals and soil of fungi which cause disease in man. *Proc. Seventh Internat. Bot. Cong., Stockholm*, p. 416, 1950.
31. AJELLO, L. Occurrence of Cryptococcus neoformans in soils. *Am. J. Hyg.*, 67: 72, 1958.
32. EMMONS, C. W. Saprophytic sources of Cryptococcus neoformans associated with the pigeon (Columba livia). *Am. J. Hyg.*, 62: 227, 1955.
33. KAO, C. J. and SCHWARZ, J. The isolation of Cryptococcus neoformans from pigeon nests. With remarks on the identification of virulent cryptococci. *Am. J. Clin. Path.*, 27: 652, 1957.
34. TAKOS, M. J. Experimental cryptococcosis produced by the ingestion of virulent organisms. *New England J. Med.*, 254: 598, 1956.
35. LITTMAN, M. L. and TSUBURA, E. Effect of degree of encapsulation upon the virulence of Cryptococcus neoformans. *Proc. Soc. Exper. Biol. & Med.* (In press.)
36. LITTMAN, M. L. Liver-spleen glucose blood agar for Histoplasma capsulatum and other fungi. *Am. J. Clin. Path.*, 25: 1148, 1955.
37. BENHAM, R. W. Cryptococci—their identification by morphology and by serology. *J. Infect. Dis.*, 57: 255, 1935.
38. UDEN, N. VAN, BRACO FORTE, M. C., JR. and GARMO, S. L. D. The occurrence of "Cryptococcus neoformans" in the equine intestinal tract. *Bull. Office internat. Epizootics*, 51: 82, 1959.
39. ANDERSON, K. and BEECH, M. Serological tests for the early diagnosis of cryptococcal infection. *M. J. Australia*, 2: 601, 1958.
40. FISCHER, J. B. and LABZOFFSKY, N. A. Preparation

- of complement fixing antigen from *Cryptococcus neoformans*. *Canad. J. Microbiol.*, 1: 451, 1955.
41. SALVIN, S. B. Quantitative studies on the serologic relationships of fungi. *J. Immunol.*, 65: 617, 1950.
 42. EVANS, E. E. An immunologic comparison of twelve strains of *Cryptococcus neoformans* (*Torula histolytica*). *Proc. Soc. Exper. Biol. & Med.*, 71: 644, 1949.
 43. SALVIN, S. B. Current concepts of diagnostic serology and skin hypersensitivity in the mycoses. *Am. J. Med.*, 27: 97, 1959.
 44. EVANS, E. E. The antigenic composition of *Cryptococcus neoformans*. I. A serologic classification by means of the capsular and agglutination reactions. *J. Immunol.*, 64: 423, 1950.
 45. ASCHNER, M., MAGER, J. and LEIBOWITZ, J. Production of extracellular starch in cultures of capsulated yeasts. *Nature, London*, 156: 295, 1945.
 46. MAGER, J. and ASCHNER, M. Biological studies on capsulated yeasts. *J. Bact.*, 53: 283, 1947.
 47. HEHRE, E. J., CARLSON, A. S. and HAMILTON, D. M. Crystalline amylose from cultures of a pathogenic yeast (*Torula histolytica*). *J. Biol. Chem.*, 177: 289, 1949.
 48. NEILL, J. M., CASTILLO, C. G., SMITH, R. H. and KAPROS, C. E. Capsular reactions and soluble antigens of *Torula histolytica* and of *Sporotrichum schenckii*. *J. Exper. Med.*, 89: 93, 1949.
 49. MAGER, J. Studies on the polysaccharides of capsulated yeasts. *Biochem. J.*, 41: 603, 1947.
 50. STANLEY, N. F. Polysaccharide haptens from *Torula histolytica*. *Australian J. Exper. Biol. & M. Sc.*, 27: 409, 1949.
 51. EVANS, E. E. and KESSEL, J. F. The antigenic composition of *Cryptococcus neoformans*. II. Serologic studies with the capsular polysaccharide. *J. Immunol.*, 67: 109, 1951.
 52. DROUHET, E. and SEGRETAIN, G. Biologie et pouvoir pathogène de *Torulopsis neoformans* (*Torula histolytica*). *Rev. path. comp. hyg. gén.*, 50: 37, 1950.
 53. EVANS, E. E. and MEHL, J. W. A qualitative analysis of capsular polysaccharides from *Cryptococcus neoformans* by filter paper chromatography. *Science*, 114: 10, 1951.
 54. EVANS, E. E. and THERIAULT, R. J. The antigenic composition of *Cryptococcus neoformans*. IV. The use of paper chromatography for following purification of the capsular polysaccharide. *J. Bact.*, 65: 571, 1953.
 55. HEIDELBERGER, M., BARKER, S. A. and REBERS, P. A. Cross-reactions of anthrax and cryptococcus-A polysaccharides in type XIV antipneumococcal horse serum. *Science*, 125: 748, 1957.
 56. EVANS, E. E., SORENSON, L. J. and WALLS, K. W. The antigenic composition of *Cryptococcus neoformans*. V. A survey of cross-reactions among strains of cryptococcus and other antigens. *J. Bact.*, 66: 287, 1953.
 57. SEELIGER, H. P. R. Das kulturell-biochemische und serologische Verhalten der *Cryptococcus*-Gruppe. *Ergebn. d. Mikrobiol., Immunitätsforsch. & Exper. Therapie*, 32: 23, 1959.
 58. UZMAN, L. L., ROSEN, H. and FOLEY, G. E. Studies on the biology of cryptococcus. VI. Amino acid composition of the somatic proteins of *Cryptococcus neoformans*. *J. Infect. Dis.*, 98: 208, 1956.
 59. GADEBUSCH, H. H. Active immunization against *Cryptococcus neoformans*. *J. Infect. Dis.*, 102: 219, 1958.
 60. GADEBUSCH, H. H. Passive immunization against *Cryptococcus neoformans*. *Proc. Soc. Exper. Biol. & Med.*, 98: 611, 1958.
 61. NEIL, J. M., SUGG, J. Y. and McCAULEY, D. W. Serologically reactive material in spinal fluid, blood, and urine from a human case of cryptococcosis (torulosis). *Proc. Soc. Exper. Biol. & Med.*, 77: 775, 1951.
 62. NEUFELD, F. Ueber die Agglutination der Pneumokokken und über die Theorien der Agglutination. *Ztschr. Hyg.*, 40: 54, 1902.
 63. EVANS, E. E., SEELIGER, H. P. R., KORNFIELD, L. and GARCIA, C. Failure to demonstrate capsular swelling in *Cryptococcus neoformans*. *Proc. Soc. Exper. Biol. & Med.*, 93: 257, 1956.
 64. KLIGMAN, A. M. Studies of the capsular substance of *Torula histolytica* and the immunologic properties of torula cells. *J. Immunol.*, 57: 395, 1947.
 65. BERGHAUSEN, O. *Torula* infection in man. *Ann. Int. Med.*, 1: 235, 1927.
 66. KESSEL, J. F. and HOLTZWART, F. Experimental studies with torula from a knee infection in man. *Am. J. Trop. Med.*, 15: 467, 1935.
 67. DIENST, R. B. *Cryptococcus histolyticus* isolated from subcutaneous tumor. *Arch. Dermat. & Syph.*, 37: 461, 1938.
 68. CARTON, C. A. Treatment of central nervous system cryptococcosis: a review and report of four cases treated with actidione. *Ann. Int. Med.*, 37: 123, 1952.
 69. LEOPOLD, S. S. Pulmonary moniliasis and cryptococcal osteomyelitis in the same patient. *M. Clin. North America*, 37: 1737, 1953.
 70. EMMONS, C. W. Isolation of *Cryptococcus neoformans* from soil. *J. Bact.*, 62: 685, 1951.
 71. ABBOT, K. H. and CUTLER, O. L. Chronic coccidioid meningitis. Review of the literature and report of seven cases. *Arch. Path.*, 21: 320, 1936.
 72. SINCLAIR, H. M. The estimation of vitamin B₁ in cerebro-spinal fluid. *Biochem. J.*, 33: 1816, 1939.
 73. ABDERHALDEN, R. and ELSAESSER, K. H. Das Vorkommen von Aneurin und Cocarboxylase im Liquor. *Arch. ges. Physiol.*, 247: 24, 1943.
 74. HIMWICH, W. A., SULLIVAN, W. T., KELLEY, B., BENARON, H. B. W. and TUCKER, B. Chemical constituents of human brain. *J. Nerv. & Ment. Dis.*, 122: 441, 1955.
 75. KREBS, H. A. The intermediary stages in the biological oxidation of carbohydrate. *Advances Enzymol.*, 3: 191, 1943.
 76. OCHOA, S. Enzymic mechanisms in the citric acid cycle. *Advances Enzymol.*, 15: 183, 1954.
 77. KLIGMAN, A. M. and WEIDMAN, F. D. Experimental studies on treatment of human torulosis. *Arch. Dermat. & Syph.*, 60: 726, 1949.
 78. SCHMIDT, E. G. and THORP, P. The effect of chemotherapeutic agents upon the growth of *Cryptococcus neoformans*. *J. Infect. Dis.*, 103: 142, 1958.
 79. COX, L. B. and TOLHURST, J. C. Human Torulosis; A Clinical, Pathological, and Microbiological Study with a Report of Thirteen Cases. Pp. 149, Melbourne, 1946. Melbourne Univ. Press.

80. WAKSMAN, S. A., SCHATZ, A. and REILLY, H. C. Metabolism and the chemical nature of *Streptomyces griseus*. *J. Bact.*, 51: 753, 1946.
81. WHIFFEN, A. J., BOHONOS, N. and EMERSON, R. L. The production of an antifungal antibiotic by *Streptomyces griseus*. *J. Bact.*, 52: 610, 1946.
82. SCHOENBACH, E. B., MILLER, J. M., GINSBERG, M. and LONG, P. H. Systemic blastomycosis treated with stilbamidine. *J. A. M. A.*, 146: 1317, 1951.
83. SCHOENBACH, E. B., MILLER, J. M. and LONG, P. H. The treatment of systemic blastomycosis with stilbamidine. *Ann. Int. Med.*, 37: 31, 1952.
84. WHITEHILL, M. R. and RAWSON, A. J. Treatment of generalized cryptococcosis with 2-hydroxystilbamidine; report of a case with apparent cure. *Virginia M. Month.*, 81: 591, 1954.
85. WARREN, W. A., STEPHENS, G. G. and CROW, E. W. Cryptococcosis; treatment of two cases with stilbamidines. *J. Kansas M. Soc.*, 58: 301, 1957.
86. LEITHOLD, S. L., REEDER, P. S. and BAKER, L. A. Cryptococcal infection treated with 2-hydroxystilbamidine in a patient with Boeck's sarcoid. *Arch. Int. Med.*, 99: 736, 1957.
87. MILLER, J. M., SCHOENBACH, E. B., LONG, P. H., SHUTTLEWORTH, J. S. and SNIDER, G. E. Treatment of infections due to *Cryptococcus neoformans* with stilbamidine. *Antibiotics & Chemother.*, 2: 444, 1952.
88. ELSON, W. O. The antibacterial and fungistatic properties of propamidine. *J. Infect. Dis.*, 76: 193, 1945.
89. SOLOTOROVSKY, M., IRONSON, E. J., GREGORY, F. J. and WINSTEN, S. Activity of certain diamidines against blastomycosis and *Candida* infections in mice. *Antibiotics & Chemother.*, 4: 165, 1954.
90. DIENST, R. B. *Cryptococcus histolyticus* isolated from subcutaneous tumor. *Arch. Dermat. & Syph.*, 37: 461, 1938.
91. BURGER, R. E. and MORTON, C. G. Torula infection: a review and report of four cases. *Surgery*, 15: 312, 1944.
92. KRAINER, L., SMALL, J. M., HEWLITT, A. B. and DENESS, T. A case of systemic torula infection with tumour formation in the meninges. *J. Neurol., Neurosurg. & Psychiat.*, 9: 158, 1946.
93. DEBUEN, S., ZIMMERMAN, L. E. and FOERSTER, H. C. Patologia ocular en la criptococcosis. *Rev. Inst. salub. y enferm. trop.*, 14: 163, 1954.
94. RAMAMURTHI, B. and ANGULI, V. C. Intramedullary cryptococcal granuloma of the spinal cord. *J. Neurosurg.*, 11: 622, 1954.
95. GOLD, W., STOUT, H. A., PAGANO, J. F. and DONOVICK, R. Amphoterics A and B, antifungal antibiotics produced by a streptomycete. 1. In vitro studies, p. 579. *Antibiotics Annual 1955-1956*. New York, 1956. Medical Encyclopedia, Inc.
96. LITTMAN, M. L., HOROWITZ, P. L. and SWADEY, J. G. Coccidioidomycosis and its treatment with amphotericin B. *Am. J. Med.*, 24: 568, 1958.
97. LITTMAN, M. L. The systemic mycoses. *Am. J. Med.*, 27: 1, 1959.
98. LITTMAN, M. L., PISANO, M. A. and LANCASTER, R. M. Induced resistance of *Candida* species to nystatin and amphotericin B, p. 981. *Antibiotics Annual 1957-58*. New York, 1958. Medical Encyclopedia, Inc.
99. MARTIN, W. J., NICHOLS, D. R., SVIEN, H. J. and ULRICH, J. A. Cryptococcosis. Further observations and experiences with amphotericin B. *Arch. Int. Med.*, 104: 4, 1959.
100. LITTMAN, M. L. and PACE, L. (Unpublished data.)
101. BRUTON, O. C. Agammaglobulinemia. *Pediatrics*, 9: 722, 1952.
102. GITLIN, D., GROSS, P. A. M. and JANEWAY, C. A. The gamma globulins and their clinical significance. *New England J. Med.*, 260: 21, 1959.
103. ENDERS, J. F. Chemical, clinical and immunological studies on the products of human plasma fractionation. x. The concentrations of certain antibodies in globulin fractions derived from human blood plasma. *J. Clin. Invest.*, 23: 510, 1944.
104. GUTMAN, A. B. The plasma proteins in disease. *Advances Protein Chemistry*, 4: 155, 1948.
105. LONGSWORTH, L. G., SHEDLOVSKY, T. and MAC-INNES, D. A. Electrophoretic patterns of normal and pathological human blood serum and plasma. *J. Exper. Med.*, 70: 399, 1939.
106. SHEDLOVSKY, T. and SCUDDER, J. A comparison of erythrocyte sedimentation rates and electrophoretic patterns of normal and pathological human blood. *J. Exper. Med.*, 75: 119, 1942.
107. TISELIUS, A. and KABAT, E. A. An electrophoretic study of immune sera and purified antibody preparations. *J. Exper. Med.*, 69: 119, 1939.
108. FISHER, M. W. Synergism between human gamma globulin and chloramphenicol in the treatment of experimental bacterial infections. *Antibiotics & Chemother.*, 7: 315, 1957.
109. MANNING, M. C., GAGLIARDI, L. A. and FISHER, M. W. The therapeutic activity of gamma globulin-chloramphenicol combinations in mouse infections due to *Salmonella*, pneumococci, *Escherichia coli* or *Pasteurella*, p. 566. *Antibiotics Annual 1957-58*. New York, 1958. Medical Encyclopedia, Inc.
110. HOLPER, J. C., RICKHER, C. J. and SYLVESTER, J. C. Ristocetin: Effect of gamma globulin on in vivo activity, p. 577. *Antibiotics Annual 1957-1958*. New York, 1958. Medical Encyclopedia, Inc.
111. WAISBREN, B. A. The treatment of bacterial infections with the combination of antibiotics and gamma globulin. *Antibiotics & Chemother.*, 7: 322, 1957.
112. KNOUF, E. G. The combined use of gamma globulin and chloramphenicol in the treatment of refractory infections. A report of ten cases, p. 585. *Antibiotics Annual 1957-1958*. New York, 1958. Medical Encyclopedia, Inc.
113. HARRIS, J. R. and SCHICK, B. The use of gamma globulin in infection refractory to antibiotics. *J. Mt. Sinai Hosp.*, 21: 148, 1954.
114. SCHONHOLTZ, G. J., BORGIA, C. A. and RITCHEY, S. J. The combined use of gamma globulin and broad-spectrum antibiotics in the treatment of osteomyelitis. A preliminary study, p. 635. *Antibiotics Annual 1958-1959*. New York, 1959. Medical Encyclopedia, Inc.
115. McALLISTER, R. M. Immunization procedures. *Pediat. Clin. North America*, p. 611, August 1957.
116. FISHER, M. W. and MANNING, M. C. Studies on the immunotherapy of bacterial infections. i. The comparative effectiveness of human γ -globulin

- against various bacterial species in mice. *J. Immunol.*, 81: 29, 1958.
117. FISHER, M. W. and MANNING, M. C. The specific antibody nature of the therapeutic action of gamma globulin in experimental bacterial infections in mice, p. 572. *Antibiotics Annual 1957-1958*. New York, 1958. Medical Encyclopedia, Inc.
118. BJØRNEBOE, M., FISCHER, E. E. and STOERCK, H. C. The effect of cortisone and adrenocorticotrophic hormone on the concentration of circulating antibody. *J. Exper. Med.*, 93: 37, 1951.
119. KASS, E. H., KENDRICK, M. I. and FINLAND, M. Effects of corticosterone, hydrocortisone and corticotropin on production of antibodies in rabbits. *J. Exper. Med.*, 102: 767, 1955.
120. SHEWELL, J. and LONG, D. A. A species difference with regard to the effect of cortisone acetate on body weight, γ -globulin and circulating antitoxin levels. *J. Hyg.*, 54: 452, 1956.
121. GERMUTH, F. G., JR., OYAMA, J. and OTTINGER, B. The mechanism of action of 17-hydroxy-11-dehydrocorticosterone (compound E) and of the adrenocorticotrophic hormone in experimental hypersensitivity in rabbits. *J. Exper. Med.*, 94: 139, 1951.
122. SCHWAB, L., MOLL, F. C., HALL, T., BREAN, H., KIRK, M., HAWN, C. V. Z. and JANEWAY, C. A. Experimental hypersensitivity in the rabbit. Effect of inhibition of antibody formation by x-radiation and nitrogen mustards on histologic and serologic sequences, and on the behavior of serum complement, following single large injections of foreign proteins. *J. Exper. Med.*, 91: 505, 1950.

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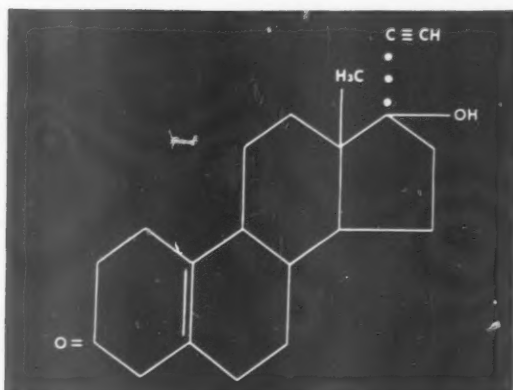
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...threatened abortion
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1. Symposium on New Steroid Compounds with Progestational Activity, Ann. New York Acad. Sc. 71:483-805 (July 30) 1958.
2. Edgren, R. A.: The Uterine Growth-Stimulating Activities of 17 α -Ethynyl-17-Hydroxy-5(10)-Estren-3-One (Norethynodrel) and 17 α -Ethynyl-19-Nortestosterone, Endocrinology 62:689 (May) 1958.
3. Rokoff, A. E.: Pages 800-805 of reference 1.
4. Tyler, E. T., and Olson, H. J.: Pages 704-709 of reference 1.
5. Kistner, R. W., Endometriosis, in Conn, H. F. (editor): Current Therapy — 1959, Philadelphia, W. B. Saunders Company, 1959, pp. 610-612.

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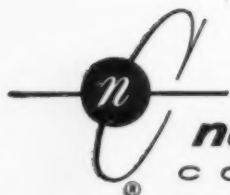
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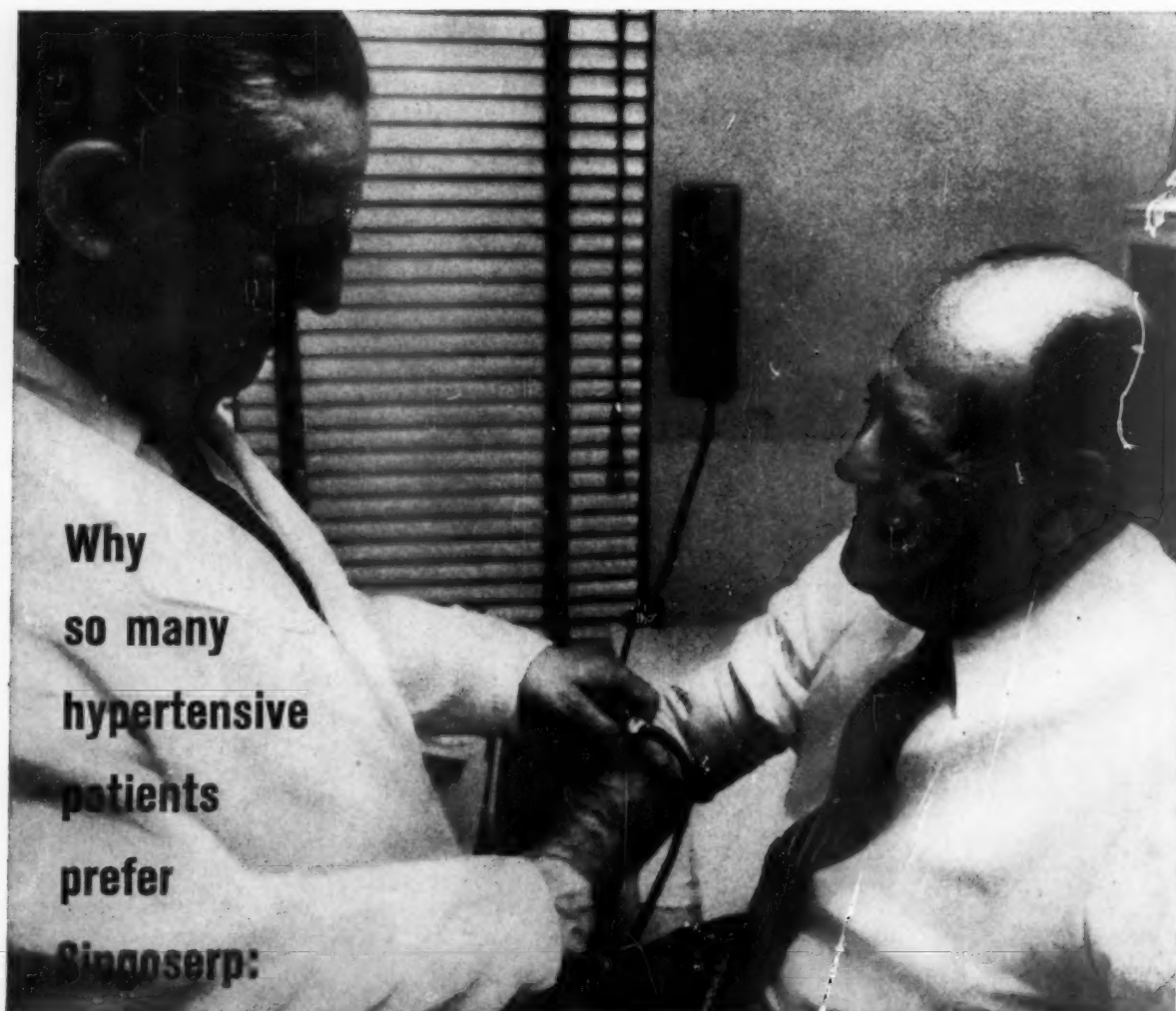
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*Herrmann, G. R., Vogelpohl, E. B., Hejtmancik, M. R., and Wright, J. C.: J.A.M.A. 169:1609 (April 4) 1959.



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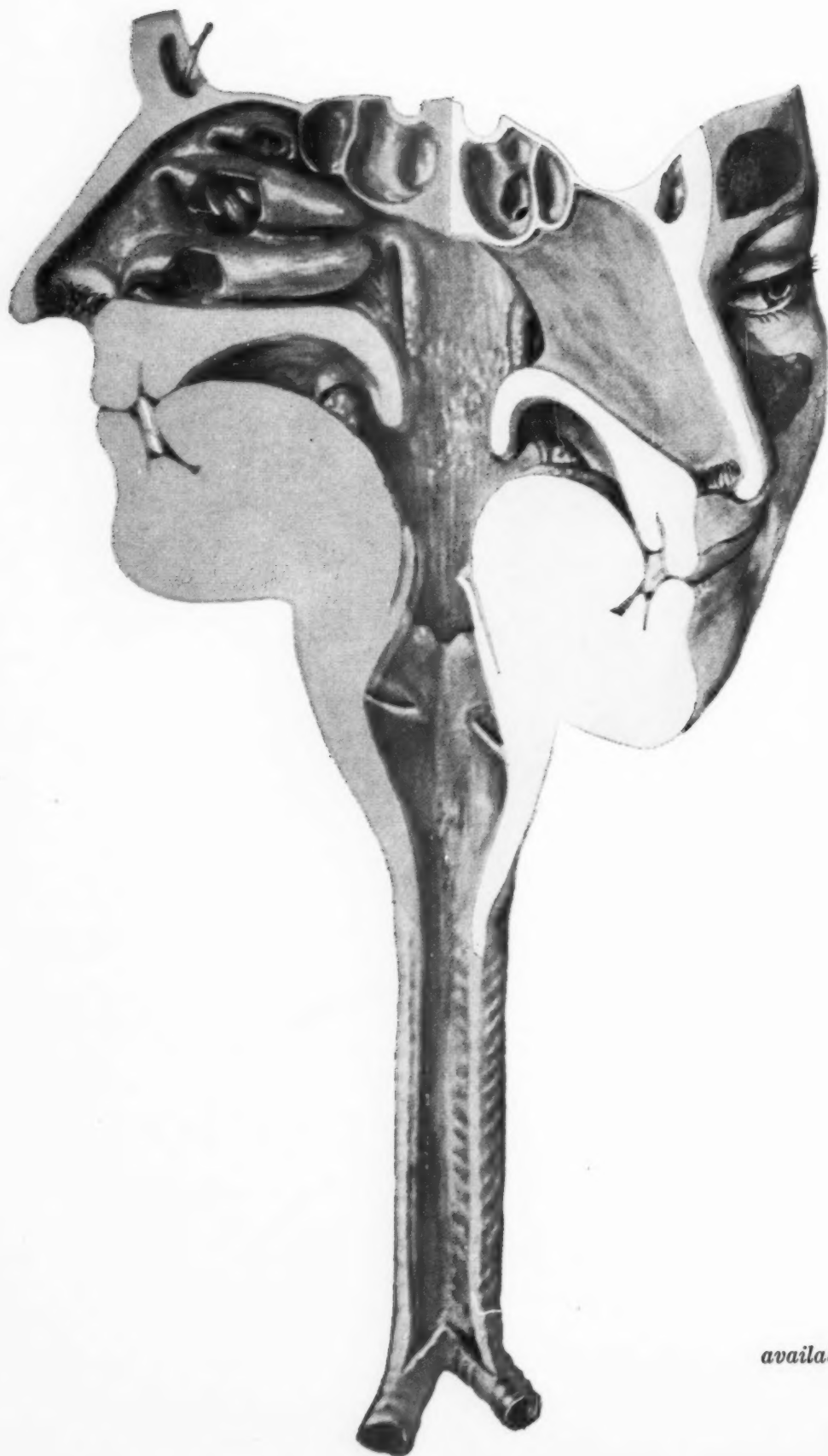
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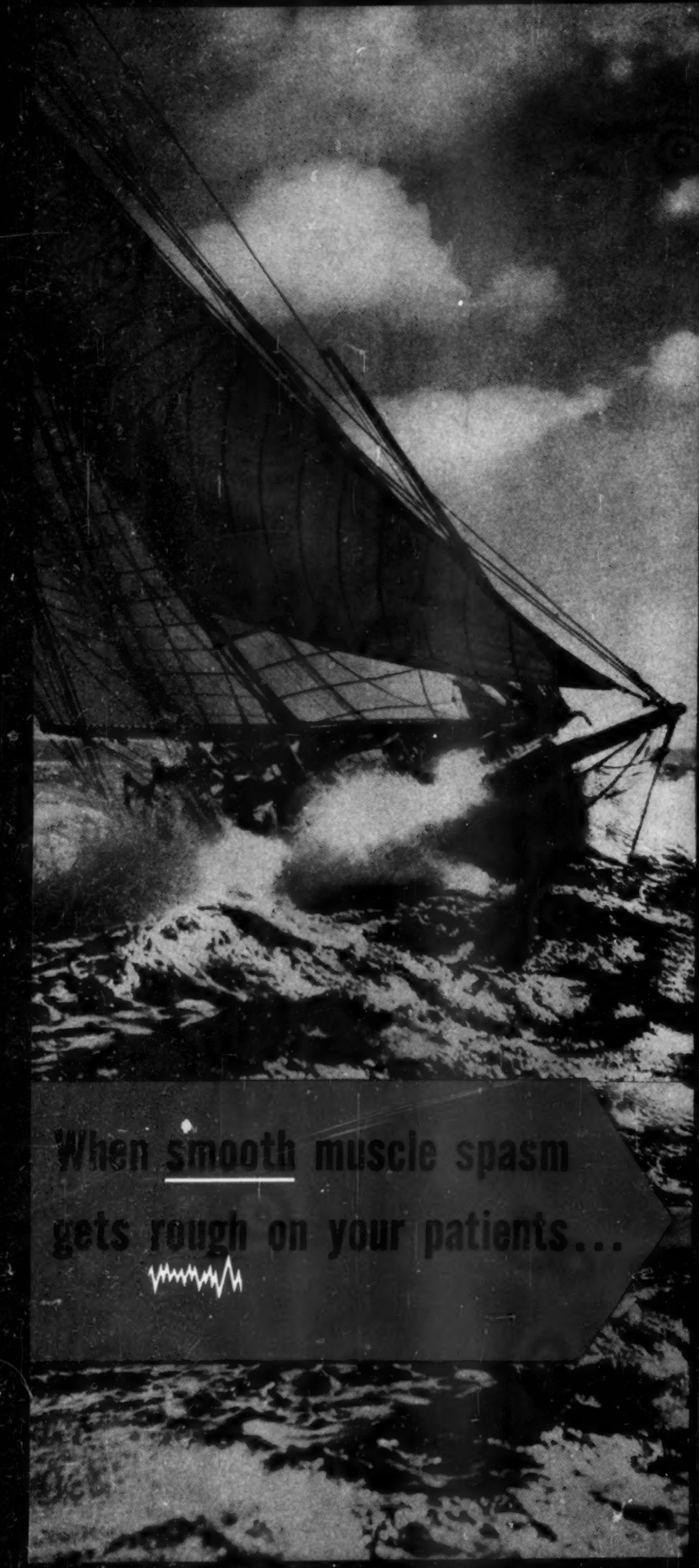
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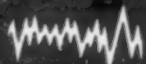


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Like oil on troubled waters


When smooth muscle spasm
gets rough on your patients...



TABLETS • CAPSULES • ELIXIR • EXTENTABS[®]

| In each Tablet, Capsule or tsp. (5 cc.) of Elixir | In each Extentab [®] |
|---|----------------------------------|
| Hyoscyamine sulfate 0.1037 mg. | 0.3111 mg. |
| Atropine sulfate 0.0194 mg. | 0.0582 mg. |
| Hyoscine hydrobromide 0.0065 mg. | 0.0195 mg. |
| Phenobarbital ($\frac{1}{4}$ gr.) 16.2 mg. | ($\frac{3}{4}$ gr.) 48.6 mg. |

*Prescribed by more physicians
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NATURAL BELLADONNA ALKALOIDS PLUS PHENOBARBITAL

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NOW

*... a new way
to relieve pain
and stiffness
in muscles
and joints*

INDICATED IN:

MUSCLE STIFFNESS

LUMBOSACRAL STRAIN

SACROILIAC STRAIN

WHIPLASH INJURY

BURSITIS

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LOW BACK PAIN

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SPRAINED BACK

"TIGHT NECK"

TRAUMATIC STRAINS
AND BRUISES

POSTOPERATIVE
MYALGIA



- Exhibits unusual analgesic properties, different from those of any other drug
- Specific and superior in relief of SOMATIC pain
- Modifies central perception of pain without abolishing natural defense reflexes
- Relaxes abnormal tension of skeletal muscle

SOMA^{T.M.}

N-isopropyl-2-methyl-2-propyl-1, 3-propanediol dicarbamate

- More specific than salicylates
- Less drastic than steroids
- More effective than muscle relaxants

SOMA has an unique analgesic action. It apparently modifies central pain perception without abolishing peripheral pain reflexes. **SOMA** is particularly effective in relieving joint pain. Patients say that they feel better and sleep better with **SOMA** than with any previously used analgesic, sedative or relaxant drug.

SOMA also relaxes muscle hypertonia, with its stresses on related joints, ligaments and skeletal structures.

ACTS FAST. Pain-relieving and relaxant effects start in 30 minutes and last 6 hours.

NOTABLY SAFE. Toxicity of **SOMA** is extremely low. No effects on liver, endocrine system, blood pressure, blood picture or urine have been reported. Some patients may become sleepy on high dosage.

EASY TO USE. Usual adult dose is one 350 mg. tablet 3 times daily and at bedtime.

SUPPLIED: Bottles of 50 white sugar-coated 350 mg. tablets.

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INTRAMUSCULAR SOLUTION

Initiation of therapy in minutes after diagnosis with new, ready-to-inject Terramycin Intramuscular Solution provides maximum, sustained absorption of potent broad-spectrum activity.

*...and for continued, compatible,
coordinated therapy*

COSA-TERRAMYCIN[®]

oxytetracycline with glucosamine

CAPSULES

Continuation with oral Cosa-Terramycin every six hours will provide highly effective antibacterial serum and tissue levels for prompt infection control.

The unsurpassed record of clinical effectiveness and safety established for Terramycin is your guide to successful antibiotic therapy.

Supply:

Terramycin Intramuscular Solution*

100 mg./2 cc. ampules

250 mg./2 cc. ampules

Cosa-Terramycin Capsules

125 mg. and 250 mg.

Cosa-Terramycin is also available as:

Cosa-Terramycin Oral Suspension — peach flavored,
125 mg./5 cc., 2 oz. bottle

Cosa-Terramycin Pediatric Drops — peach flavored,
5 mg./drop (100 mg./cc.), 10 cc. bottle with plastic calibrated dropper

Complete information on Terramycin Intramuscular Solution and Cosa-Terramycin oral forms is available through your Pfizer Representative or the Medical Department, Pfizer Laboratories.

*Contains 2% Xylocaine[®] (lidocaine), trademark of Astra Pharmaceutical Products, Inc.

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"the commonest gynecologic office problem"

"VULVOVAGINITIS, CAUSED BY TRICHOMONAS VAGINALIS, CANDIDA ALBICANS, Haemophilus vaginalis, or other bacteria, is still the commonest gynecologic office problem . . . cases of chronic or mixed infection are often extremely difficult to cure." Among 75 patients with vulvovaginitis caused by one or more of these pathogens, TRICOFURON IMPROVED cleared symptoms in 70; virtually all were severe, chronic infections which had persisted despite previous therapy with other agents. "Permanent cure by both laboratory and clinical criteria was achieved in 56. . . ."

Essey, J. E.: Am. J. Obst. 77:155, 1959

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Improved

- Swiftly relieves itching, burning, malodor and leukorrhea
- Destroys Trichomonas vaginalis, Candida (Monilia) albicans, Haemophilus vaginalis
- Achieves clinical and cultural cures where others fail
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2 steps to lasting relief:

1. **POWDER** for weekly insufflation in your office. MICOFUR[®], brand of nifuroxime, 0.5% and FUROXONE[®], brand of furazolidone, 0.1% in an acidic water-dispersible base.
2. **SUPPOSITORIES** for continued home use each morning and night the first week and each night thereafter—especially during the important menstrual days. MICOFUR 0.375% and FUROXONE 0.25% in a water-miscible base.

*Rx new box of 24 suppositories with applicator
for more practical and economical therapy.*

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"The best results . . ."

"The best results . . . in both clinical and electrocardiographic response, were observed with a combination of meprobamate and pentaerythritol tetranitrate [EQUANITRATE]. . . ." Russek¹ so reported using double-blind methods in an important new study of pentaerythritol tetranitrate, a placebo, meprobamate, and EQUANITRATE.

EQUANITRATE reduces the frequency and severity of attacks and controls angina-triggering emotions.

Supplied: EQUANITRATE 10 (200 mg. meprobamate, 10 mg. pentaerythritol tetranitrate), white oval tablets, vials of 50. EQUANITRATE 20 (200 mg. meprobamate, 20 mg. pentaerythritol tetranitrate), yellow oval tablets, vials of 50.

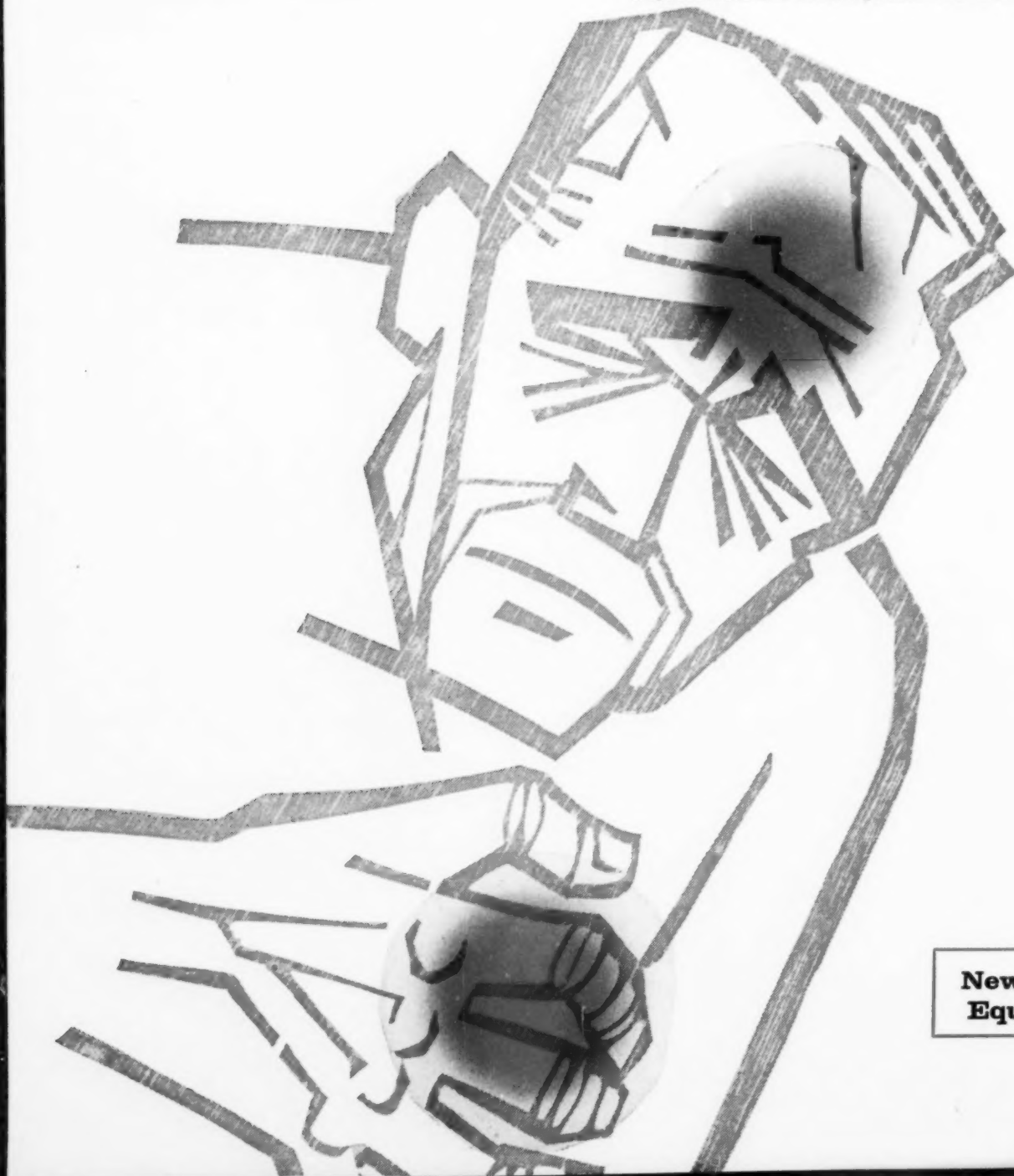
1. Russek, H.I.: Am. J. Cardiol. 3:547 (April) 1959.

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relieves painful muscle spasm, improves mobility, facilitates rehabilitation...

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Chlorzoxazone*

PARAFLEX provides effective skeletal muscle relaxation for about 6 hours with a 1- to 2-tablet dose. It relieves pain and stiffness and improves function in a wide variety of orthopedic, arthritic, and rheumatic disorders. It may be used alone or with other agents indicated in the management of skeletal muscle spasm. It is especially valuable when used in conjunction with physiotherapy ● and other rehabilitative procedures. Side effects are rare, almost never require discontinuance of therapy.

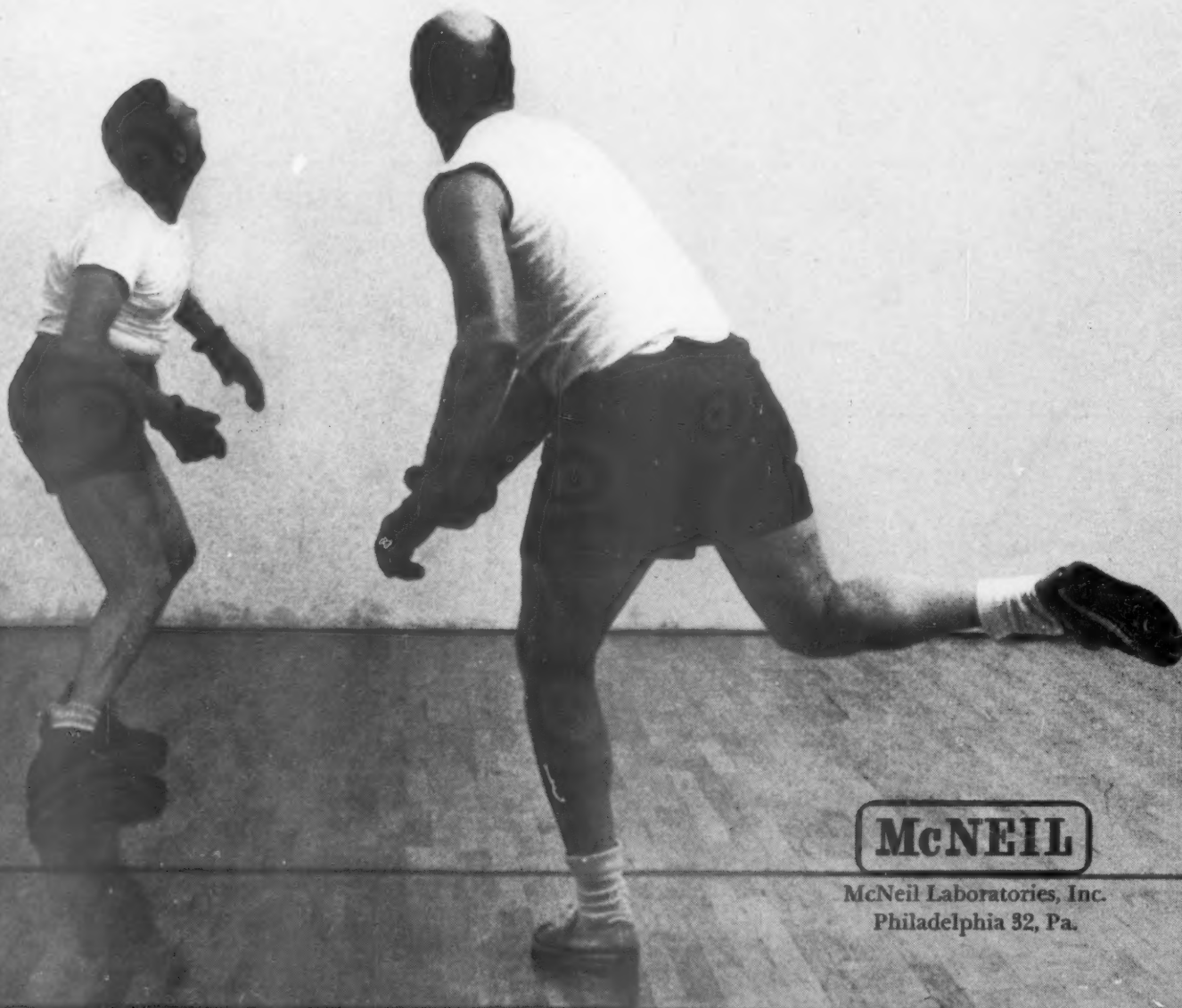
Dosage: ADULTS—1 to 2 tablets three or four times a day.

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289A89



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Specific in Depression

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Produce remission or improvement in
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Act effectively in all types of depression

Afford equally good results in severe
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Achieve therapeutic benefit with minimal risk of
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Indications for Tofrānil include:

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.....not a MAO inhibitor

does not

Inhibit monoamine oxidase either in
brain or liver with its associated risks

Produce dangerous potentiation of other
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Act by producing undesirable central
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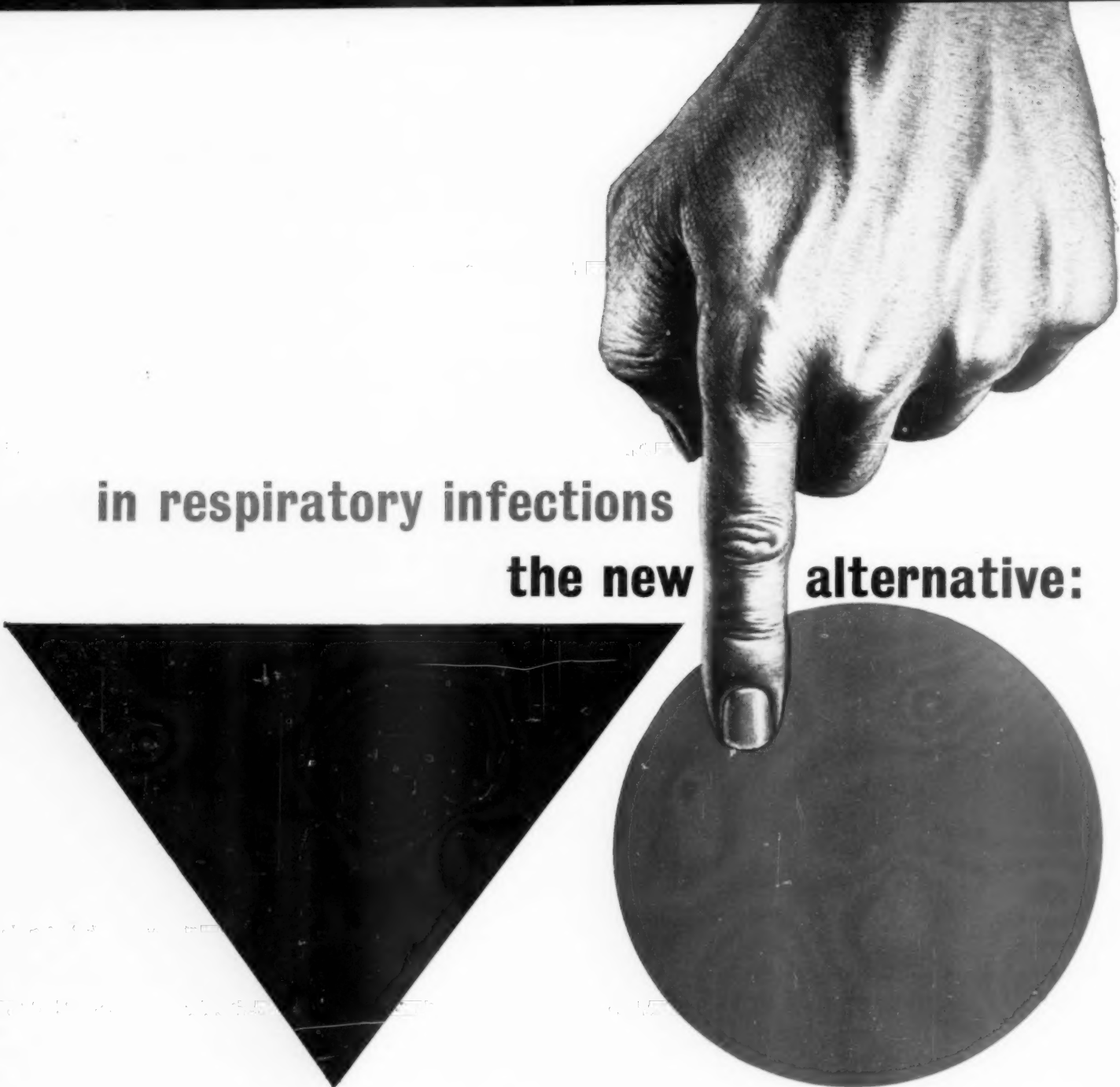
The efficacy of Tofranil is attested by more than 50
published reports and confirmed by clinical experi-
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Detailed Literature Available on Request.



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A black and white photograph of a hand pointing its index finger down towards a dark, solid circle. To the left of the circle is a dark, solid triangle. The hand is positioned at the top right of the frame, with the finger pointing towards the center of the circle. The triangle and circle are positioned below the text.

**in respiratory infections
the new alternative:**

In 25 years, the antibacterials have progressed from the status of heroic therapy to "universal" medication. This has brought into focus certain unexpected problems relating both to bacterial and to host response.

Shifts in bacterial flora—particularly of the gastrointestinal, as well as the respiratory and urinary tracts—pose entirely new therapeutic problems. The emergence of resistant strains of bacteria creates still another hazard. Also, anaphylactic reactions often hamper critically needed therapy.

While the question of bacterial mutations and patient sensitivity is undergoing continual intensive study, the immediate clinical need is for a new anti-infective alternative.

**The fastest-growing
antibacterial
bibliography:**

1. W. P. Boger, *Antibiotics Annual 1954-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 48.
2. B. A. Koechlin, W. Kern and R. Engelberg, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 22-31, Feb. 1959. 3. S. Ross, J. R. Pulg and E. A. Zarembo, *Antibiotics Annual 1954-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 56. 4. E. H. Townsend, Jr. and A. Borgstedt, *Antibiotics Annual 1954-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 64. 5. J. D. Young, Jr., W. S. Kiser and O. C. Beyer, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 53-56, Feb. 1959. 6. B. H. Leming, Jr., C. Flanigan, Jr. and B. R. Jennings, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 32-39, Feb. 1959. 7. T. D. Michael, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 57-60, Feb. 1959. 8. W. A. Leff, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 44-48, Feb. 1959. 9. J. C. Ellis, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 61-64, Feb. 1959. 10. M. J. Mosely, Jr., *J. Nat. M. A.*, 51:258, July 1959. 11. S. Guss and A. J. Spiro, *Pediatric Conference*, 2:14, Mar. 1959. 12. H. P. Ironson and C. Patel, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 40-43, Feb. 1959. 13. R. J. Schnitzer and W. F. DeLorenzo, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 17-21, Feb. 1959. 14. R. J. Schnitzer, W. F. DeLorenzo, E. Grunberg and R. Russomanno, *Proc. Soc. Exper. Biol. & Med.*, 99:421, Nov. 1958. 15. W. F. DeLorenzo and R. Russomanno, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 14-16, Feb. 1959. 16. B. Fast and E. Bochni, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 3-10, Feb. 1959. 17. W. F. DeLorenzo and A. M. Schumacher, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 11-13, Feb. 1959. 18. O. Brandman, C. Oyer and R. Engelberg, *J. M. Soc. New Jersey*, 56:24, Jan. 1959. 19. J. F. Glenn, J. R. Johnson and J. H. Semans, *Antibiotic Med. & Clin. Therapy*, 6:(Suppl. 1), 49-52, Feb. 1959. 20. L. O. Randall, R. E. Bagdon and R. Engelberg, *Toxicol. & Appl. Pharmacol.*, 1:28, Jan. 1959. 21. S. M. Finegold, Z. Kudihoff, H. O. Kendall and V. E. Kvinge, *Ann. New York Acad. Sci.*, 82:11, 44-50, 1959. 22. W. J. Grace, *ibid.*, p. 51. 23. L. E. Skinner, *ibid.*, pp. 57-60. 24. C. W. Daeschner, *ibid.*, pp. 64-70. 25. S. W. Levy, *ibid.*, pp. 80-83. 26. M. M. Cahn and E. J. Draper, *ibid.*, pp. 92-104. 27. G. A. Moore, *ibid.*, pp. 61-63. 28. J. C. Ellis, *ibid.*, pp. 52-56. 29. W. S. Kiser, O. C. Beyer and J. D. Young, *ibid.*, pp. 105-113. 30. B. H. Leming, Jr. and C. Flanigan, Jr., *ibid.*, pp. 31-39. 31. R. E. Bagdon, L. O. Randall and W. A. Leff, *ibid.*, pp. 3-9. 32. W. F. DeLorenzo and R. J. Schnitzer, *ibid.*, pp. 10-17. 33. G. Carroll, *Discussant, ibid.*, pp. 110-113. 34. S. Krugman, *Discussant, ibid.*, pp. 78-79. 35. E. H. Townsend, Jr. and A. Borgstedt, *ibid.*, pp. 71-79. 36. T. D. Michael, *ibid.*, pp. 40-43. 37. A. Thill, to be published. 38. J. C. Ellis, *Mil. Med.*, in press. 39. B. Wolach, *Colorado GP*, 1:4, 1959. 40. H. L. Rosenthal and L. Jud, *J. Lab. & Clin. Med.*, 54:461-466, Sept. 1959. 41. R. E. Rav, *Case Rep. Child. Mem. Hosp., Chicago*, 17:443-446, May 1959. 42. P. Rentchnick and J. Lazler, *Schweiz. med. Wochschr.*, 89:894-898, Aug. 22, 1959. 43. J. Leng-Lévy, J. David-Chausse, P. Gibaud and J. Bottin, *J. méd. Bordeaux*, 136:161, 713-720, June 1959. 44. B. H. Leming, Jr. and C. Flanigan, Jr., *Scientific Exhibit, Annual Meeting of the American Medical Association, Atlantic City, N. J.*, June 1959. 45. J. C. Ellis, *ibid.*, 48. O. Thalhammer (University Pediatric Clinic, Vienna, Austria), paper presented at the International Congress of Infectious Pathology, Milan, Italy, May 6-10, 1959. 46. R. Schuppil (Director, University Dermatological Clinic, Basle, Switzerland), *ibid.*, 50. S. Rummelhardt (First University Surgical Clinic, Vienna, Austria), *ibid.*, 51. M. Rineti (Institute of Surgical Pathology, University of Parma), *ibid.*, 52. M. Rentsch (University Pediatric Clinic, Berne, Switzerland), *ibid.*, 53. N. Quattrin (Cardarelli Hospital, Naples, Italy), *ibid.*, 54. H. Ptasnik (Department of Gynecology and Obstetrics, St. Francis Hospital, Bielefeld, Germany), *ibid.*, 55. E. Picha (First University Gynecological Clinic, Vienna, Austria), *ibid.*, 56. R. Neimeier (University Gynecology Clinic, Basle, Switzerland), *ibid.*, 57. G. Moustardier (Faculty of Medicine and St. Andrew's Hospital, Bordeaux, France), *ibid.*, 58. S. T. Madsen (Bergen, Norway), *ibid.*, 59. W. P. Boger, *ibid.*, 60. P. Buenger (Medical Department, Heidelberg General Hospital, Langenhorn, Hamburg, Germany), *ibid.*

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in chronic constipation...

and especially that associated with
the irritable bowel syndrome



DECHOTYL

TABLETS*

provides physiologic support until function returns

*T.M. for AMES trapezoid-shaped tablet.



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safe, gentle transition to normal bowel function

DECHOTYL provides gentle stimulation of the bowel and helps restore normal consistency of the intestinal contents to gradually re-establish normal bowel function in your chronically constipated patients.

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By emulsifying fat globules, desoxycholic acid aids the digestive action of the fat-splitting
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tion and the movement of the intestinal contents.

Diocetyl Sodium Sulfosuccinate (50 mg.) is a wetting agent which lowers surface
tension and aids the penetration of intestinal fluids into the fecal mass, providing a moist
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EFFECTIVE: Bile influences the constitution as well as the movement of the intes-
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1. Moser, K. M.: J.A.M.A. 167:1695 (Aug. 2) 1958.
2. Clifton, E. E.: J. Am. Geriatrics Soc. 6:118, 1958.
3. Sussman, B. J., and Fitch, T. S.: J. A. M. A. 167:1705 (Aug. 2) 1958.
4. Singher, H. O., and Chapple, R. V.: Clin. Med. 6:439 (March) 1959.

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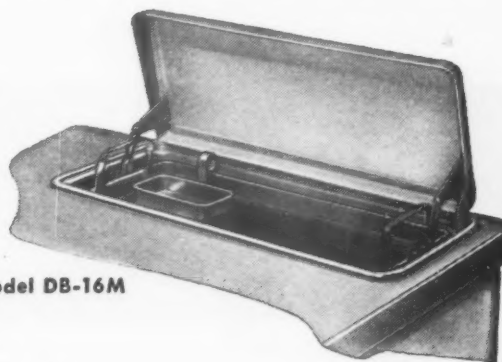
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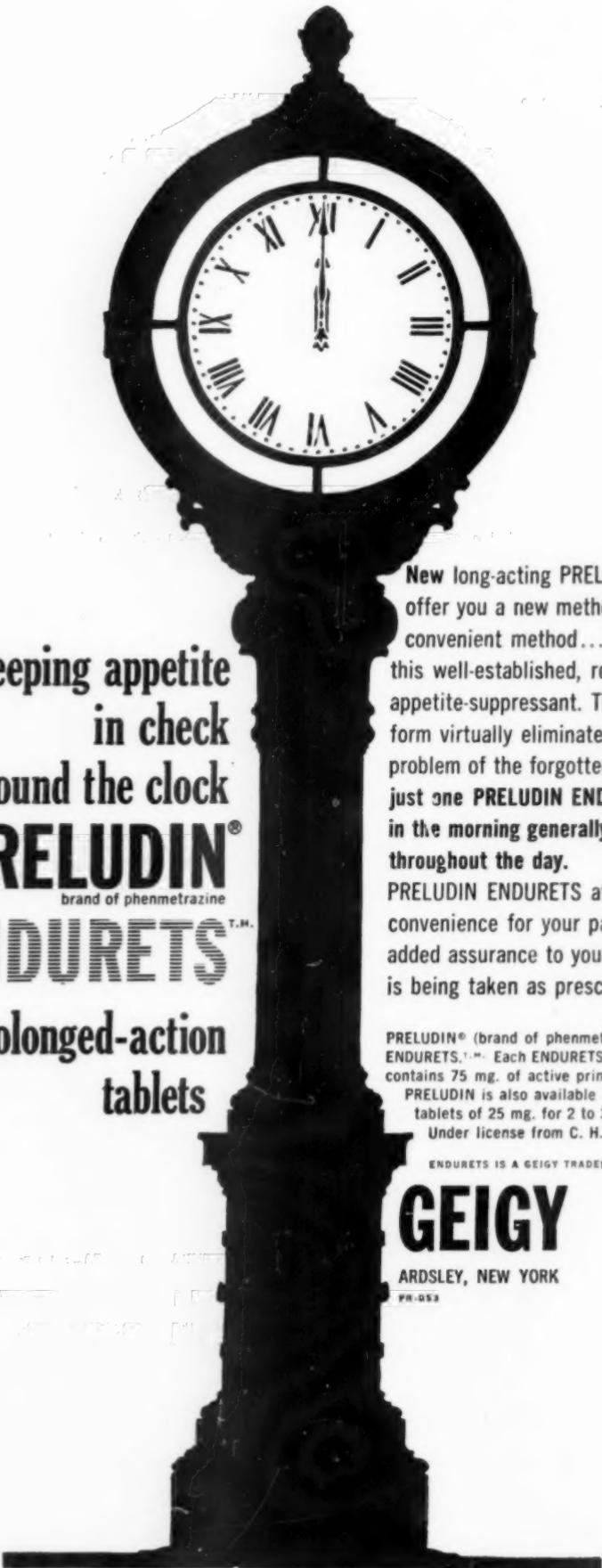
Amsco's large Office Pressure-Steam Sterilizer, 613-R Dynaclave, or 8" Square Autoclave, Cat. No. 8816, can be conveniently located on work counter of the double cabinet.



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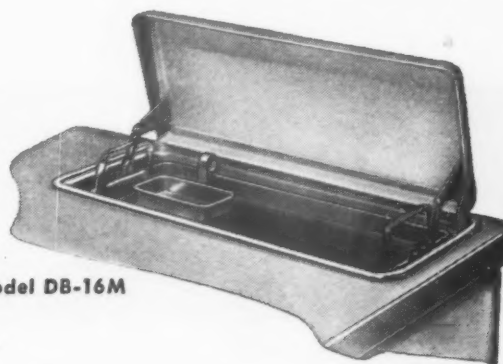
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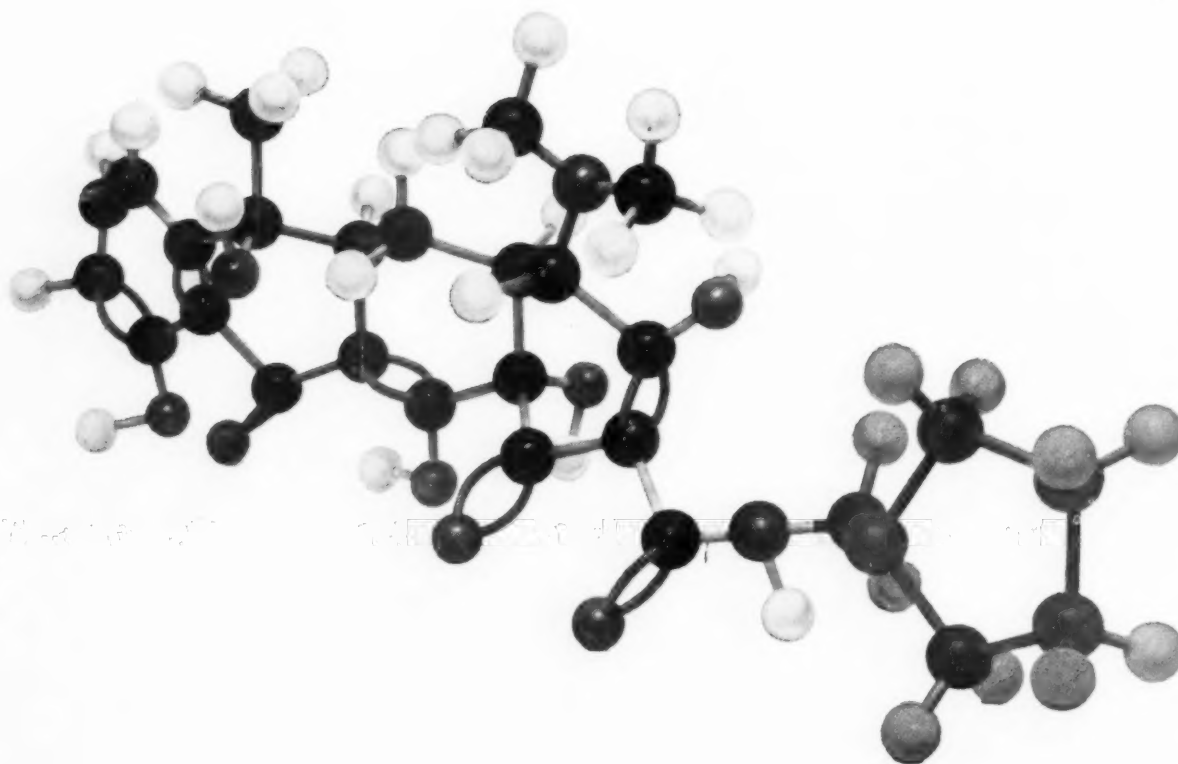
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SYNTETRINTM

N-(PYRROLIDINOMETHYL) TETRACYCLINE

a new¹
improved
broad-spectrum antibiotic
for parenteral administration



SYNTETRIN — a new synthetic derivative of tetracycline — has these attributes of significant value in therapy:

- effective antibacterial activity is sustained — *even at its lowest blood levels* — throughout therapy
- total antibiotic activity of SYNTETRIN I.M. more than twice that with tetracycline phosphate complex I.M. over a 24-hour period
- highly soluble over the entire physiological pH range (2,500 times more soluble than tetracycline) resulting in more efficient absorption from intramuscular sites than other tetracycline I.M. preparations

An important advantage of SYNTETRIN is that the *lowest* blood levels reached before ensuing daily injections are either maintained or increased. This means that antibiotic levels will not drop below those required to inhibit certain pathogens during the course of therapy. Successive blood level peaks generally rise after repeated injections.

Parenteral SYNTETRIN is recommended for initial therapy in infections caused by tetracycline-sensitive organisms in:

1. Patients who require frequent force-feeding or special diets based on milk, which interfere with antibiotic absorption.
2. Patients with diseases causing absorption difficulties.
3. Patients unable to take anything by mouth.

I. Gottstein, W. J.; Minor, W. F., and Cheney, L. C.: J. Am. Chem. Soc. **81**:1198, 1959.

for intramuscular injection

SYNTETRIN I.M.

N-(PYRROLIDINOMETHYL) TETRACYCLINE WITH XYLOCAINE®* FOR INTRAMUSCULAR USE

Supplied in dry-fill single dose vials:

SYNTETRIN I.M. '350' contains:

| | |
|---------------------|---------|
| SYNTETRIN | 350 mg. |
| Lidocaine | 40 mg. |
| Ascorbic Acid | 300 mg. |

SYNTETRIN I.M. '150' contains:

| | |
|---------------------|---------|
| SYNTETRIN | 150 mg. |
| Lidocaine | 40 mg. |
| Ascorbic Acid | 300 mg. |

*Xylocaine is the registered trademark of Astra Pharmaceutical Products, Inc. for lidocaine.

for intravenous infusion

SYNTETRIN I.V.

N-(PYRROLIDINOMETHYL) TETRACYCLINE FOR INTRAVENOUS INFUSION

Supplied in dry-fill vials, each containing: SYNTETRIN 700 mg.
Ascorbic Acid 500 mg.

Detailed information on indications, dosage and precautions is contained in package insert; or, write to Medical Director, Bristol Laboratories Inc., New York, New York.





Before Esidrix:
Weight 176 lbs.

27 pounds lost in 19 days; ascites and

RECORD OF TREATMENT (At a leading New York City hospital. Photos used with permission of the patient.)

| Date | 3/3 | 3/4 | 3/5 | 3/6 | 3/7 | 3/8 | 3/9 | 3/10 | 3/11 | 3/12 | 3/13 | 3/14 | 3/15 | 3/16 | 3/17 | 3/18 | 3/19 | 3/20 | 3/21 | 3/22 | 3/23 |
|-----------------|-----|-----|-----------------------|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Weight (pounds) | 178 | 176 | 170 | 169 | 167 | 159 | 158 | 158 | 157 | 153 | 155 | 155 | 156 | 154 | 153 | 154 | 153 | — | — | 151 | 149 |
| Rx | M* | | Esidrix 50 mg. b.i.d. | | | | | | | | | | | | | | | | | | |

*Mercurial diuretic



Esidrix^{T.M.}

(hydrochlorothiazide CIBA)

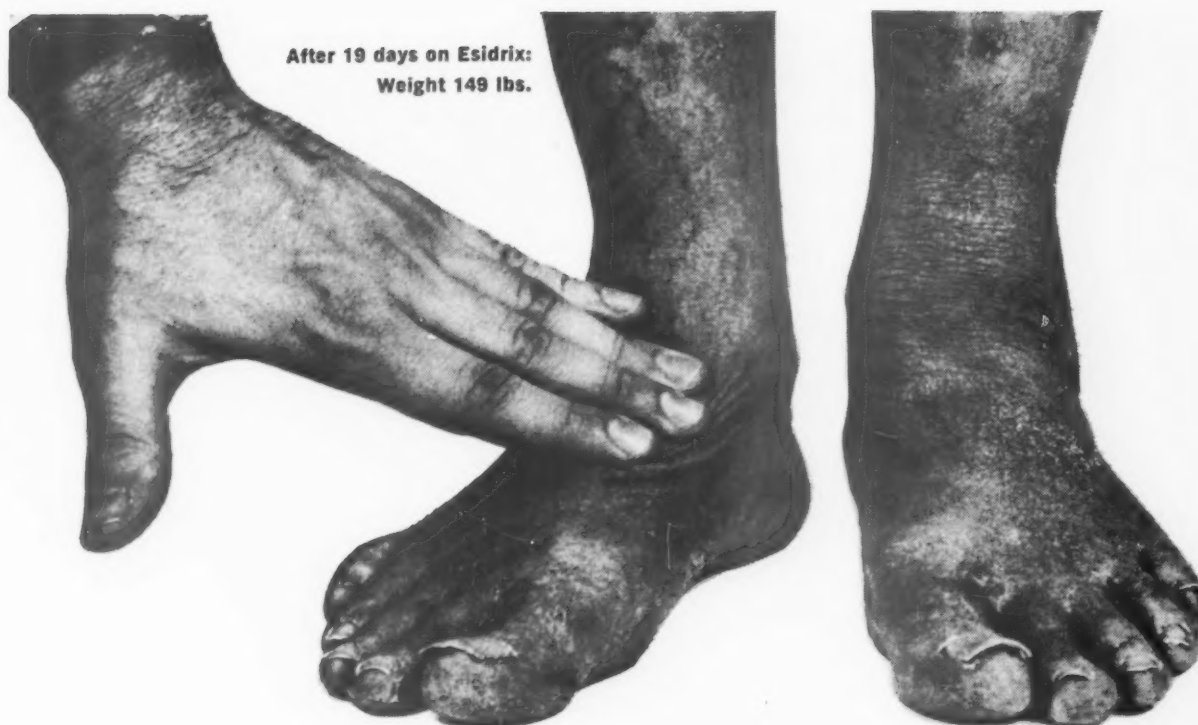
pre-eminently effective whenever diuresis is desired

*Indicated in: congestive heart failure . . . nephrosis and nephritis
. . . toxemia of pregnancy . . . premenstrual edema . . . edema of
pregnancy . . . steroid-induced edema . . . edema of obesity*

Supplied: Esidrix Tablets,
25 mg. (pink, scored)
and 50 mg. (yellow, scored);
bottles of 100 and 1000.

C I B A
SUMMIT, N. J.





pedal edema reduced with Esidrix

H. K., 44 years old, was admitted to the hospital on 3/3/59 with complaints of swollen abdomen, swelling of both legs and exertional dyspnea. These symptoms had been intensifying over a three-week period. The patient's history included heavy drinking since the age of 18, and one prior admission to the hospital in 1954 with ascites and pedal edema. Diagnosis, at that time, was Laennec's cirrhosis, and the patient responded well to a regimen of diuretics, salt restriction and multivitamins. There was no recurrence up to that leading to his current admission.

2/2714MK



Clinical findings worthy of note: Eyes — conjunctivae and sclerae slightly icteric. Chest—diaphragm elevated. Abdomen — girth enlarged, definite fluid wave. Liver palpated 4 fingerbreadths below the costal margin; no other palpable viscera. Extremities—pedal edema (4+).

The patient is well developed and not in acute distress. Blood pressure, 140/80 mm. Hg; pulse, 112/min.; respiration, 20/min. Impression: Laennec's cirrhosis—decompensated.

Treatment: Mercurial diuretic on 3/3 and 3/4, followed by Esidrix, 50 mg. b.i.d., from 3/5 to 3/23 when patient signed out of hospital. Esidrix induced copious diuresis resulting in almost complete disappearance of edema.

RONIACOL

FOR INTERMITTENT CLAUDICATION

...FOR PERIPHERAL VASOSPASM

DIRECT VASCULAR RELAXATION. RONIACOL relieves pain, extends range of walking and raises activity-tolerance by direct dilation of peripheral vessel musculature, thus increasing blood flow throughout the extremities.¹⁻³

NO KNOWN CONTRAINDICATIONS. RONIACOL, unlike sympathetic blocking agents, may be used safely for prolonged periods in peripheral vasospasm of any etiology, even in patients with coronary disease. Further, RONIACOL (nicotinic alcohol) is converted at the cellular level to the pure vitamin (nicotinic acid); side effects are absent, negligible or inconsequential.¹⁻⁴

References: (1) R. O. Gilhespy, *Brit. M. J.*, 1:207, 1957. (2) M. M. Fisher and H. E. Tebrock, *New York J. Med.*, 53:65, 1953. (3) C. M. Castro and L. de Soldati, *Angiology*, 4:165, 1953. (4) W. Redisch and O. Brandman, *Angiology*, 1:312, 1950. (5) G. Kagan, *Lancet* 2:53, 1959.

RONIACOL, scored 50-mg tablets, bottles of 100, 500, and 1000. RONIACOL ELIXIR, 50 mg of Ronicol per teaspoonful (5 cc), bottles of 16 oz and 1 gal.

RONIACOL®—brand of beta-pyridyl carbinol.

ROCHE LABORATORIES • Division of Hoffmann-La Roche Inc • Nutley 10, N. J.





Striking relief
from **LOW BACK PAIN**
and **DYSMENORRHEA**

THE FIRST TRUE "TRANQUILAXANT"
Trancopal



Here is what you can expect when you prescribe

Case Profile*

A 28-year-old married woman, a secretary in a booking agency, complained of severe and consistent pain and cramps in the abdomen during her menstrual periods. Psychologically, she described the first two days as "climbing the walls." Menarche occurred at age 13. She has a regular twenty-eight day menstrual cycle and a four day menstrual period.

Trancopal was given in a dose of 100 mg. four times a day for the first two days of the four day period. In addition to the relief of the dysmenorrhea she also noticed disappearance of a "bloated feeling" that had previously annoyed her. She has now been treated with Trancopal for one and one-half years with excellent results. Other medication, such as codeine or aspirin with codeine, had relieved the pain, but the patient had had to stay home. Because her father is a physician, many commercial preparations had been tried prior to Trancopal, but no success had been achieved.

Before taking Trancopal this patient missed one day of work every month. For the past year and a half she has not missed a day because of dysmenorrhea.

for dysmenorrhea
and premenstrual tension



Trancopal[®]

THE FIRST TRUE "TRANQUILAXANT"

for low back pain



Case Profile*

A 42-year-old truck driver and mover injured his back while moving a piano. The pain radiated from the sacral region down to the region of the Achilles tendon on the right side. X-rays for ruptured disc revealed nothing pertinent. The day of the injury he was given Trancopal immediately after the physical examination. Although 100 to 200 mg. three times a day were prescribed, the patient on his own responsibility increased the dosage of Trancopal to 400 mg. three times a day. This dosage was continued for three days and then gradually reduced over a ten day period. During this time, the patient continued to drive his truck. The muscle spasm was completely controlled and no apparent side effects were noted.

For the past six months, the patient has continued to take Trancopal 100 to 200 mg. as needed for muscle spasm, particularly during strenuous days.

**Clinical Reports on file at the Department of Medical Research, Winthrop Laboratories.*

Turn page for complete listings of Indications and Dosage.

THE FIRST TRUE "TRANQUILAXANT" *Trancopal*

potent MUSCLE RELAXANT

effective TRANQUILIZER

- In musculoskeletal disorders, effective in 91 per cent of patients.¹
- In anxiety and tension states, effective in 89 per cent of patients.¹
 - Low incidence of side effects (2.3 per cent of patients). Blood pressure, pulse rate, respiration and digestive processes are unaffected by therapeutic dosage. It does not affect the hematopoietic system or liver and kidney function.
 - No gastric irritation. Can be taken before meals.
- No clouding of consciousness, no euphoria or depression.

Indications ¹⁻⁶

Musculoskeletal:

Low back pain
(lumbago, etc.)
Neck pain (torticollis)
Bursitis
Rheumatoid arthritis
Osteoarthritis
Disc syndrome

Fibrositis
Ankle sprain, tennis
elbow
Myositis
Postoperative muscle
spasm

Psychogenic:

Anxiety and tension
states
Dysmenorrhea
Premenstrual tension
Asthma
Angina pectoris
Alcoholism

Now available in two strengths:

NEW
STRENGTH ▶



Trancopal Caplets®,
100 mg. (peach colored, scored), bottles of 100.



Trancopal Caplets,
200 mg. (green colored, scored), bottles of 100.

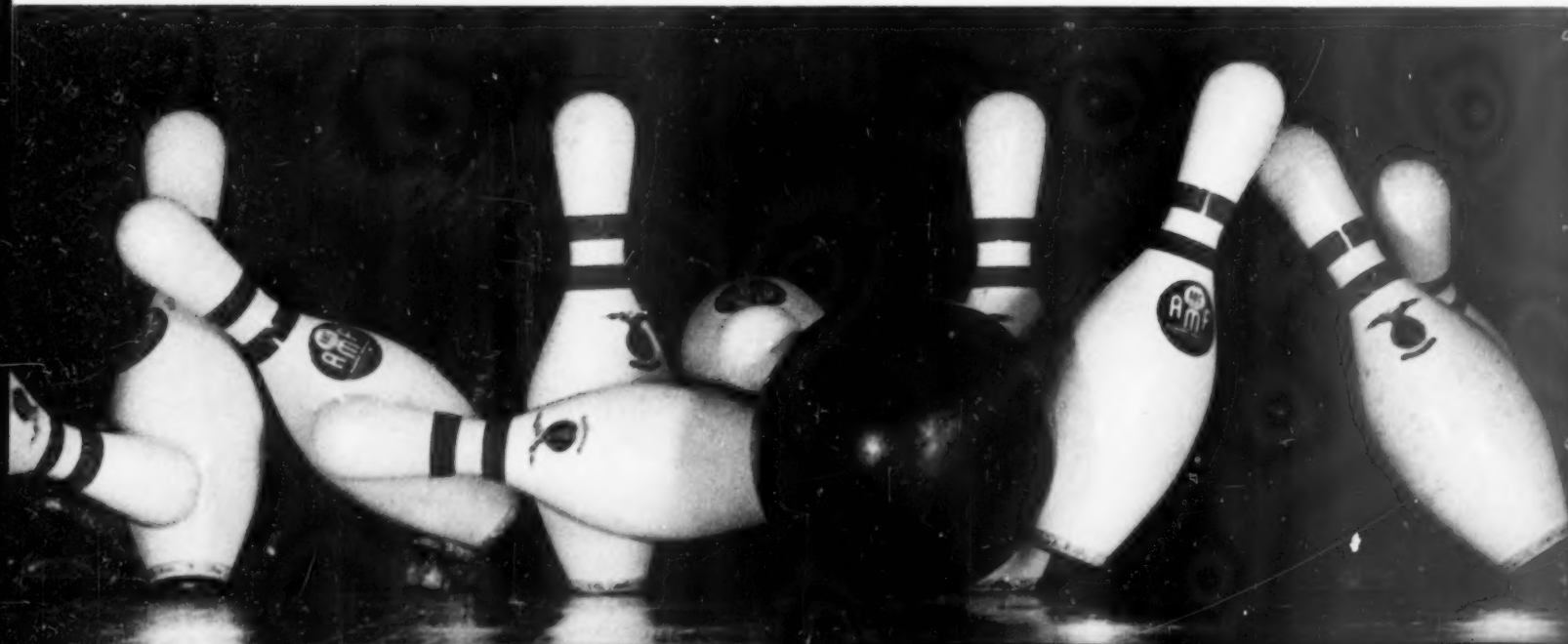
Dosage: Adults, 100 or 200 mg. orally three or four times daily. Relief of symptoms occurs in from fifteen to thirty minutes and lasts from four to six hours.

Winthrop

LABORATORIES
New York 18, N. Y.

References: 1. Collective Study, Department of Medical Research, Winthrop Laboratories. 2. Lichtman, A. L.: New developments in muscle relaxant therapy, *Kentucky Acad. Gen. Pract. J.* 4:28, Oct., 1958. 3. Lichtman, A. L.: Relief of muscle spasm with a new central muscle relaxant, chlormezanone (Trancopal), Scientific Exhibit, Meeting of the International College of Surgeons, Miami Beach, Fla., Jan. 4-7, 1959. 4. Ganz, S. E.: Clinical evaluation of a new muscle relaxant (chlormethazanone), *J. Indiana M. A.* 52:1134, July, 1959. 5. Mullin, W. G., and Epifano, Leonard: Chlormezanone, a tranquilizing agent with potent skeletal muscle relaxant properties, *Am. Pract. Digest Treat.* 10:1743, Oct., 1959. 6. Shanaphy, J. F.: Chlormezanone (Trancopal) in the treatment of dysmenorrhea: a preliminary report, *Current Therap. Res.* 1:59, Oct., 1959.

Trancopal (brand of chlormezanone) and Caplets, trademarks reg. U.S. Pat. Off. 1408M Printed in U.S.A.



A quinidine of choice in atrial fibrillation, flutter, premature contractions, auricular tachycardia.

DOSAGE: see PDR for dosage, etc.

SUPPLIED: Bottles of 30, 100, 250.

1. Bellet, S.; Finkelstein, D., and Gilmore, H.: A.M.A. Archives Int. Med. 100:750, 1957.

2. Bellet, S.: Amer. Heart J. 56:479, 1958.

3. Finkelstein, D.: Penn. Med. J. 61:1216, 1958.



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for samples and literature, write . . .

WYNN PHARMACAL CORPORATION

5119 West Stiles Street, Philadelphia 31, Pa.

also available:

INJECTABLE QUINAGLUTE

10 cc. Multiple Dose Vials, 0.08 Gm.

Quinidine Gluconate per cc.

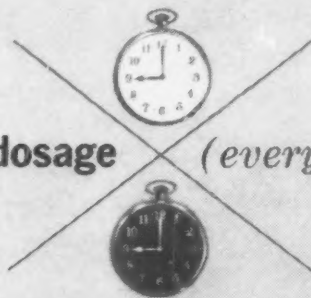
*U. S. Patent 2895881

no longer need patients be
denied quinidine benefits in
cardiac arrhythmias because of g.i. distress

QUINAGLUTE DURA-TAB[®] S.M.

provides well tolerated quinidine gluconate (ten times
as soluble as quinidine sulfate) in Sustained Medication* form

oral b.i.d. dosage (every 12 hours)



Each Quinaglute Dura-Tab S.M. dose maintains uniform effective plasma levels up to 12 hours. Night dosage unnecessary. Safer, more efficient — no valleys where arrhythmias tend to recur.¹⁻³



SPONTIN®

(Ristocetin, Abbott)

A STATISTICAL REVIEW* OF THREE HUNDRED THIRTY-THREE CASES

*Records of Medical Department, Abbott Laboratories, North Chicago, Illinois

SPONTIN (Ristocetin, Abbott) is a new antibiotic discovered and developed at Abbott Laboratories.

Its two components, A and B, have been isolated from the fermentation of a new species of *Nocardia lurida*. Both are active against gram-positive bacteria and mycobacteria. A mycete was isolated from the sample collected from the Gods, Colorado Springs. No other culture was found. The same antibiotic has been used.

The chemical characteristics of ristocetins are not completely known, though they are known to be phenolic substances containing phenolic groups. Ristocetin A and B are molecules with molecular weights of about 4000. They have good stability in a pH range of blood. SPONTIN is a lyophilized preparation, derived from material, representing ristocetins A and B.

Antimicrobial activity against gram-positive organisms, SPONTIN is more effective than other available antibiotics.

Against pneumococci (except *S. pneumoniae*) the antibiotic is bactericidal at the concentration which inhibits the growth. It also kills the bacteria.

This observation is for the major staphylococci. However, staphylococci have been tested at concentrations as low as 0.001 minimum inhibitory concentration to produce a bacteriostatic effect. For this reason, SPONTIN is used for the treatment of staphylococcal and enterococcal infections.

Cultures of staphylococcus aureus, which are resistant to other antibiotics

have been shown to be sensitive to SPONTIN. There has been no case reported in which a staphylococcal or enterococcal strain has exhibited a

SPONTIN

resistance to SPONTIN. There has been no case reported in which a staphylococcal or enterococcal strain has exhibited a

Antimicrobial Properties. In its action against gram-positive cocci or bacteria, SPONTIN is notably more effective than other antibiotics available.

and streptococci of enterococci are variably bactericidal. The concentration which inhibits the growth of organisms

holds true for strains of staphylococci which required a concentration higher than the recommended concentration to effect. It is for this reason that the recommended dosage of staphylococcal infections.

Staphylococcus aureus, to other antibiotics, has been no case reported in which a staphylococcal or enterococcal strain has exhibited a resistance to SPONTIN.

It is noted that the antimicrobial activity of SPONTIN is enhanced by gamma globulin. This is supported by the *in vivo* activity of SPONTIN, which is sometimes greater than that from the *in vitro* activity.

Investigators* have recorded a clinical response following administration of SPONTIN. Studies have shown a bacteriostatic effect on the part of the organism. Satisfactory results may be expected. SPONTIN requires up to 25 mg. for inhibition. The following table shows sensitivities of different strains of the major pathogenic

Summary and Conclusions

Major use has been treating staphylococcal infections. Of the total 333 cases, approximately one-third was treated for pneumonia; of these over 80% were either cured or improved. About 70% of these pneumonias were caused by staphylococci.

The next largest group included 46 patients with subacute bacterial endocarditis. About 50% of these infections were identified as staphylococcal and a further 15% as enterococcal. Other infections included 38 cases of septicemia, 32 abscesses and 24 patients with osteomyelitis.

The administration of SPONTIN brought about a cure in 60% of all the cases reviewed and improvement in a further 17%.

Side-effects were seldom troublesome when a daily dose of 2 Gm. was not exceeded. The incidence rose as the dosage was increased. The most disturbing side-effect after administration of SPONTIN has been neutropenia. However, in all instances this has responded to either discontinuance of medication or reduction in dose.

pH range of blood and tissue fluids. SPONTIN is a lyophilized preparation of groups.

REFLECTION ON CORTICOTHERAPY:

CRITERIA

Tablet size?

Potency per milligram?

Often these are valued.

*But the only
criterion of genuine
clinical significance is
the ratio of
desired effects
to undesired effects.*

Hence...

Medrol^{*}

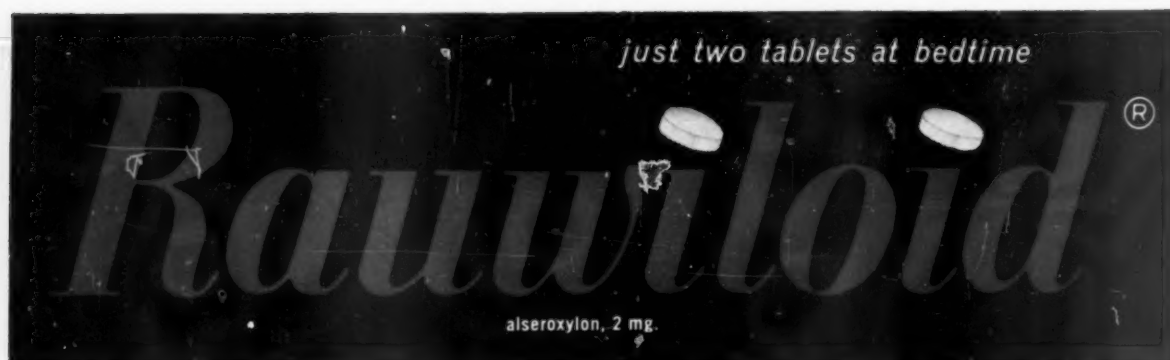
the corticosteroid that hits the
disease, but spares the patient

**Upjohn**

THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

^{*}TRADEMARK, REG. U. S. PAT. OFF.—METHYLPREDNISOLONE, UPJOHN

Classic Treatment in Hypertension*



When more potent drugs are
needed, prescribe one of the *con-
venient single-tablet combinations*

Rauwiloid® + Veriloid®

alseroxylon 1 mg. and alkavervir 3 mg.
or

Rauwiloid® + Hexamethonium

alseroxylon 1 mg. and hexamethonium
chloride dihydrate 250 mg.

Many patients with severe hypertension can be main-
tained on Rauwiloid alone after desired blood pres-
sure levels are reached with combination medication.


*** *Because***

RAUWILOID provides effective Rauwolfia
action virtually free from serious side effects
... the smooth therapeutic efficacy of Rauwiloid
is associated with a lower incidence of certain
unwanted side effects than is reserpine...and
with a lower incidence of depression. Toler-
ance does not develop.

RAUWILOID can be initial therapy for most
hypertensive patients... Dosage adjustment is
rarely a problem.



Northridge, California



cough due to colds or allergy

Rx

AMBENYL® EXPECTORANT

for quick, effective relief

- Antiallergic, antispasmodic, demulcent
- Reduces bronchial spasm and congestion
- Helps to thin mucus and facilitates expectoration

Each fluidounce of AMBENYL EXPECTORANT contains:

| | |
|---|---------|
| Ambodryl® hydrochloride (bromodiphenhydramine hydrochloride, Parke-Davis) | 24 mg. |
| Benadryl® hydrochloride (diphenhydramine hydrochloride, Parke-Davis) | 56 mg. |
| Dihydrocodeinone bitartrate | 1/6 gr. |
| Ammonium chloride | 8 gr. |
| Potassium guaiacolsulfonate | 8 gr. |
| Menthol | q.s. |
| Alcohol | 5% |

Supplied: Bottles of 16 ounces and 1 gallon.

Dosage: Every three or four hours — adults, 1 to 2 teaspoonfuls; children, 1/2 to 1 teaspoonful.

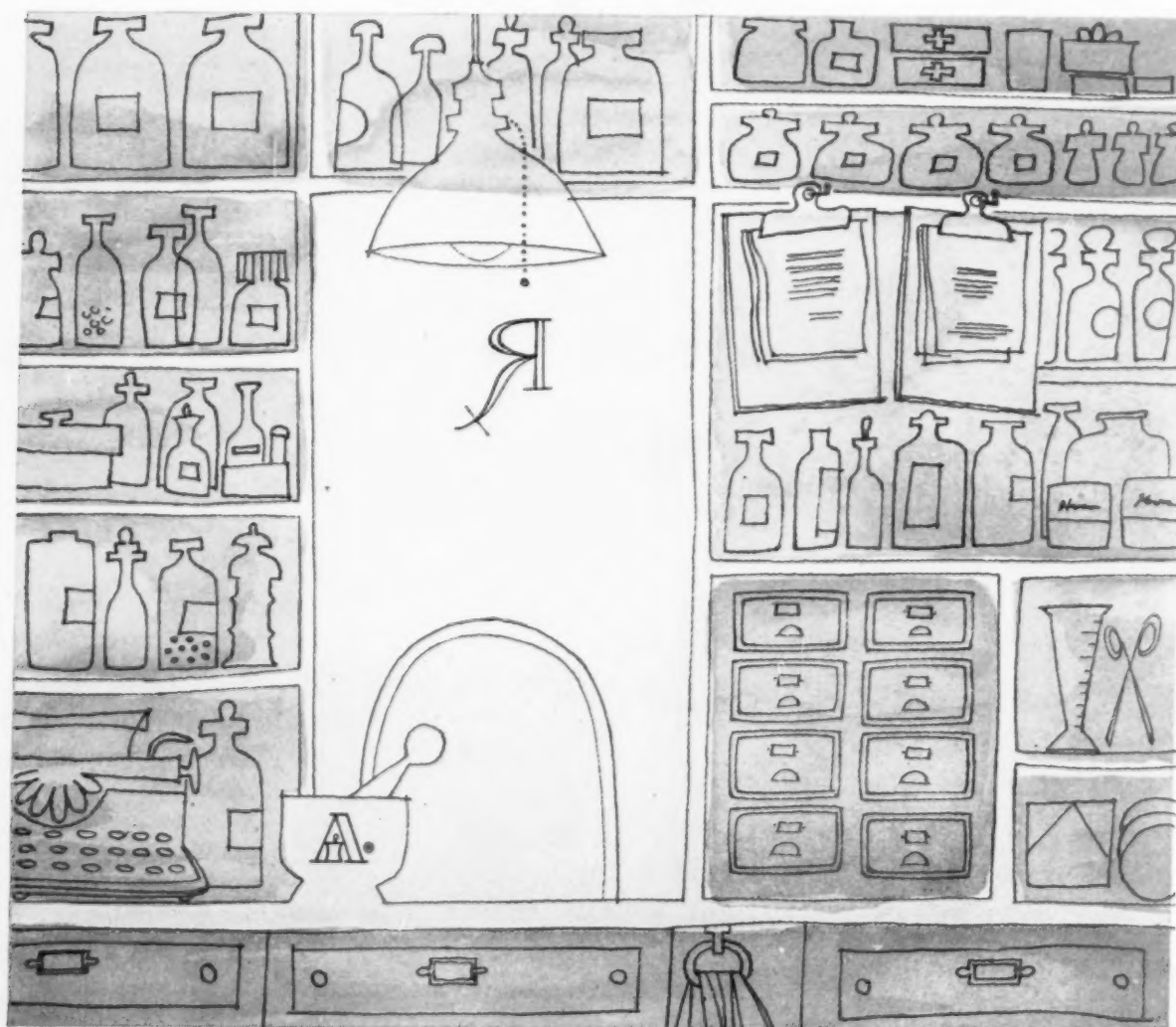


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DETROIT 32, MICHIGAN

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is used so widely and so often...stocked by so many leading pharmacies...regarded throughout the world as the pioneer in thyroid standardization and the original standard of comparison for all thyroid preparations

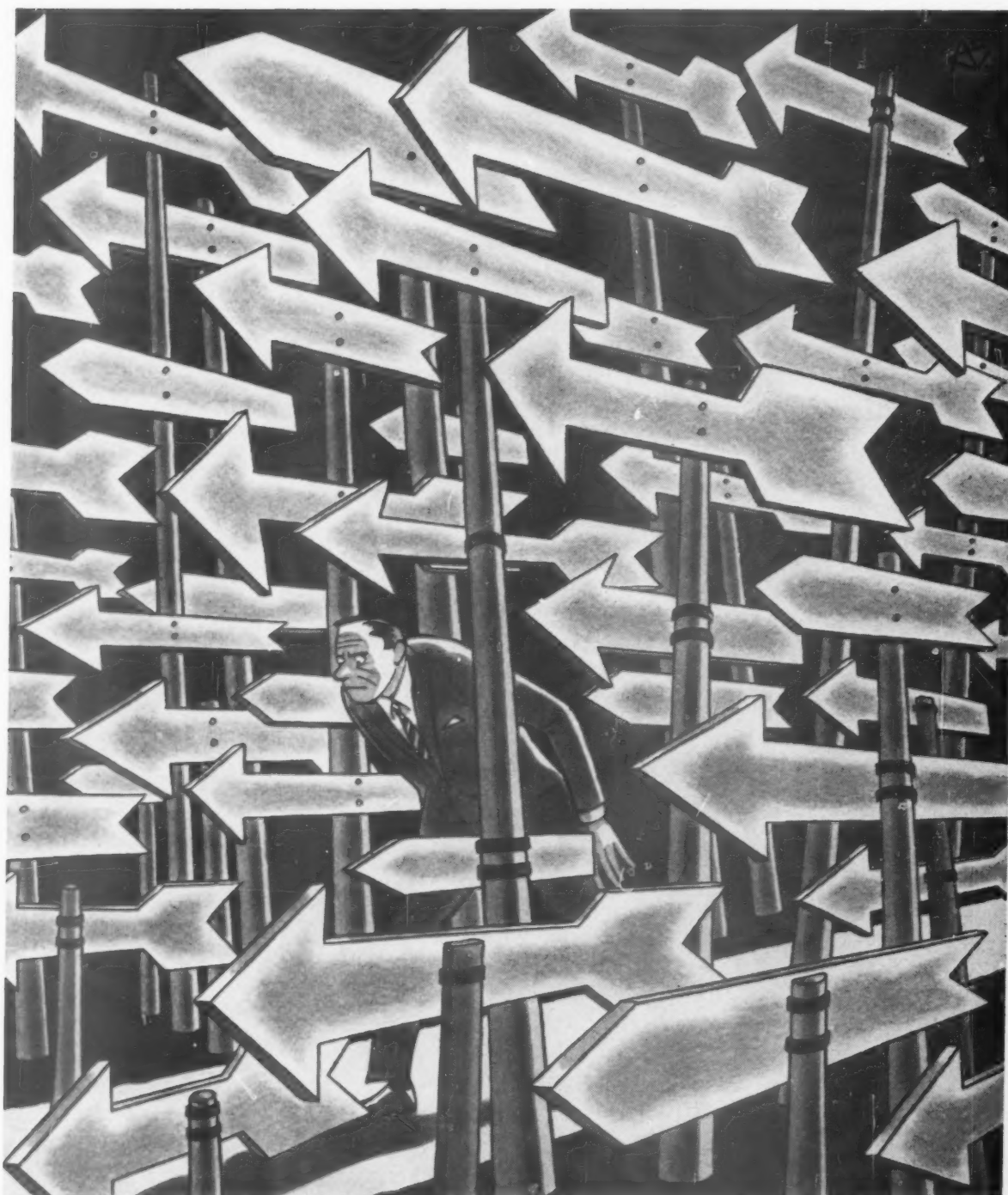
ALWAYS SPECIFY ARMOUR THYROID



ARMOUR PHARMACEUTICAL COMPANY

KANKAKEE, ILLINOIS

Armour Means Protection



The constraint of rigid management

Many diabetics on insulin live highly restricted lives. They may not miss or delay a meal; they must neither over-work nor under-exercise for fear of complications.

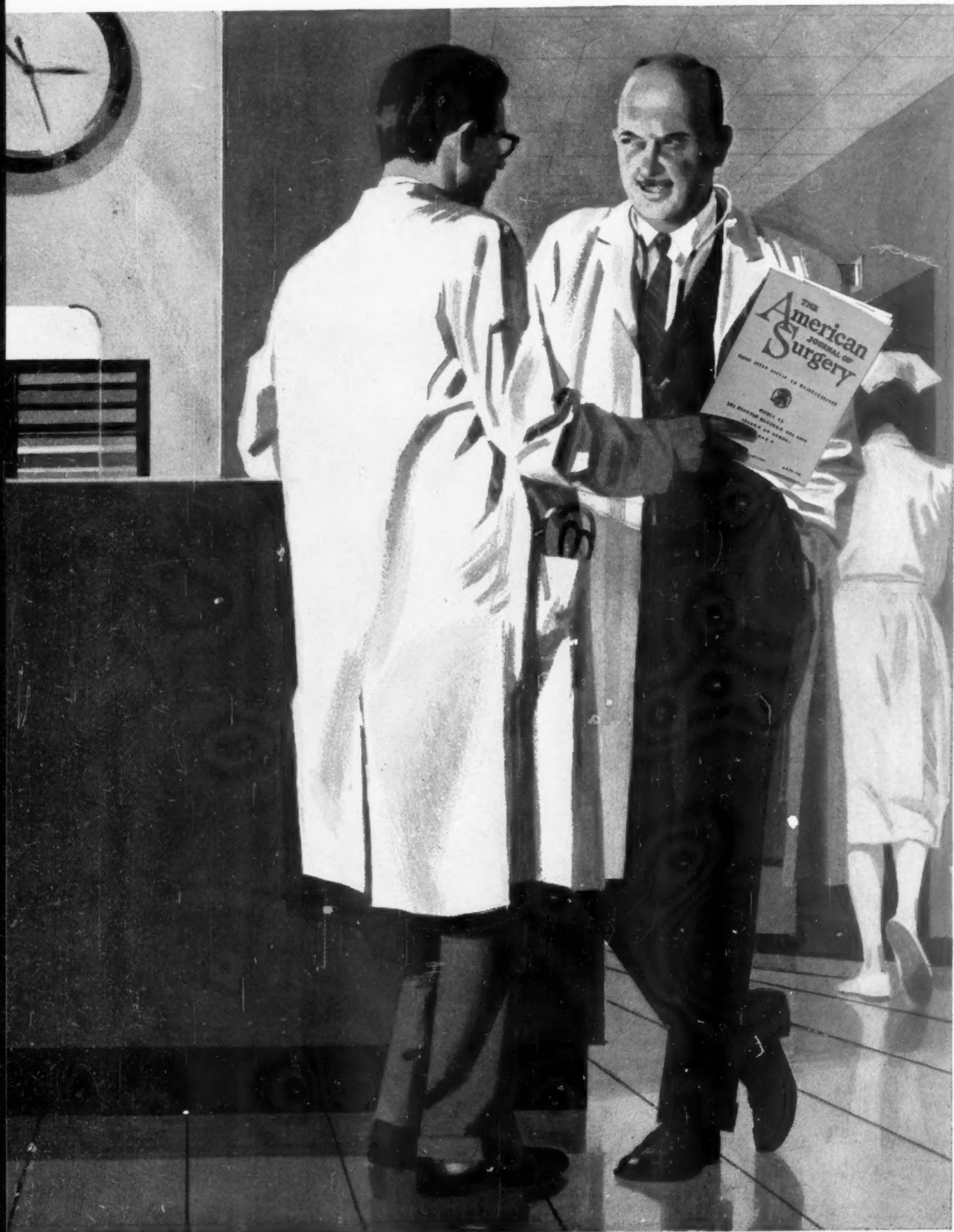
For 3 out of 4 of these patients, Orinase* offers better control and an easier, more normal life. Because Orinase controls diabetes effectively and *smoothly* in responsive patients, they can enjoy a new freedom. And some diabetics, who cannot be managed on Orinase alone, do best on *combined* Orinase-insulin therapy.

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now...ulcer
patients
sleep
through
the night
without
pain or
discomfort

new TRAL[®] 75^{mg.} Gradumet[®]

(Hexocyclium Methylsulfate in Long-Release Dose Form*, Abbott)

new anticholinergic dosage form aimed specifically at controlling nocturnal secretion

Ever wished for an anticholinergic with built-in *timing*, to have most of its effect at peak hours of secretion? New TRAL Gradumet, 75 mg., is just that—releases its anticholinergic continuously, over 8 to 12 hours, yet its action is controlled so that maximum release occurs in the critical 2:00 to 4:00 a.m. peak period of nighttime secretion and discomfort. Clinical studies show that in patients suffering from chronic and recurrent peptic ulcer—nocturnal pain is promptly relieved in up to 90% of these patients. • As shown in 48-hour gastric analyses, TRAL Gradumet, 75 mg., diminishes acidity for prolonged periods up to 12 hours. TRAL Gradumet, 75 mg., reduces volume of nocturnal secretion substantially, permitting patients to obtain a full night's sleep without intermittent medication. Because TRAL Gradumet employs no enteric coatings, its controlled metering of drug release is never altered by changes in gastric pH... gastrointestinal motility... or enzymatic activity. New TRAL Gradumet, 75 mg. (List No. 6949), is supplied at all pharmacies, in bottles of 50 and 500.



*PATENT APPLIED FOR

new ataractic-anticholinergic combination for
medical management of functional bowel disease

new **TRALCYON**TM

(Hexocyclium Methylsulfate with Ectylurea, Abbott)

combining a highly selective anticholinergic
with one of the most subtle and safe calmatives

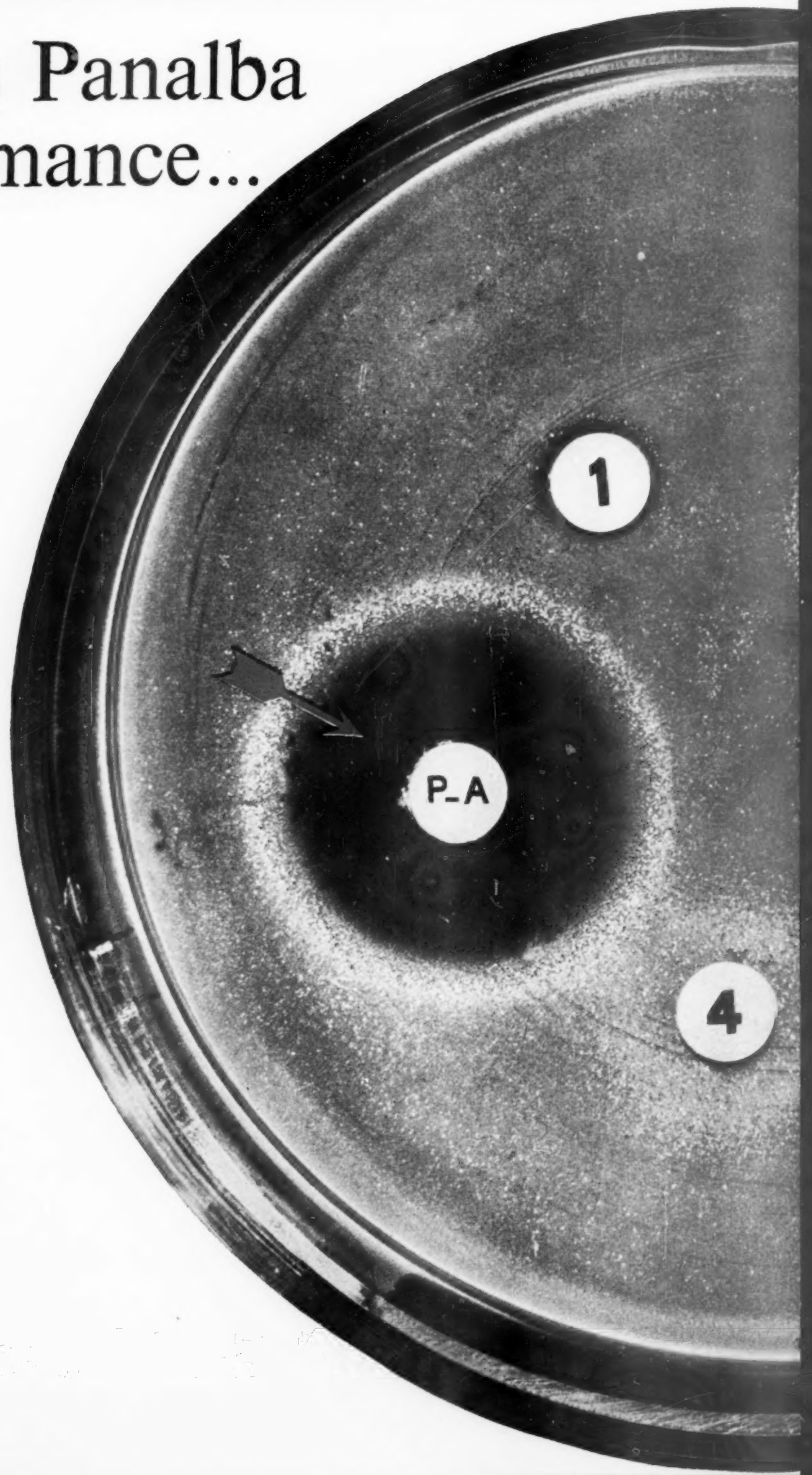
New TRALCYON Filmtabs are indicated for the management of gastrointestinal dysfunction caused or aggravated by anxiety or tension. Disorders such as spastic and irritable colon, intestinal colic, hypermotility of the stomach or intestines and anxiety neuroses with vague gastrointestinal complaints respond well to treatment with Filmtab TRALCYON. When psychogenic factors aggravate an established organic lesion such as peptic ulcer, ulcerative colitis, or biliary tract disease, TRALCYON will usually produce superior benefit to the administration of an anticholinergic agent alone. TRALCYON Filmtabs (List No. 6980), each containing TRAL[®], 25 mg., with ectylurea, 300 mg., are available at all pharmacies, in bottles of 50 and 500.



*Filmtab—Film Sealed Tablets, Abbott; U. S. Pat. No. 2,881,055.

912246

This is Panalba
performance...





in sinusitis

... into a mixed culture of the four organisms commonly involved in sinusitis ... *Str. hemolyticus*, *D. pneumoniae*, *H. influenzae* and *Staph. aureus* (in this case a resistant strain) ... we introduce the five most frequently used antibiotics.

Twenty-four hours later (in this greatly enlarged photograph), note that only *one* of the five leading antibiotics has stopped *all* the organisms, including the resistant staph! This is Panalba.

In your next patient with sinusitis ... in *all* your patients with potentially-serious infections ... provide this extra protection with your prescription:

Dosage—1 or 2 capsules
3 or 4 times a day.
Supplied—Capsules containing
Panmycin phosphate equivalent
to 250 mg. tetracycline
hydrochloride, and 125 mg.
Albamycin as novobiocin
sodium, in bottles of 16 and 100.
Now available: new Panalba
Half-Strength Capsules in
bottles of 16 and 100.

Panalba*

(Panmycin* Phosphate plus Albamycin*)

The broad-spectrum
antibiotic of
first resort

Upjohn

The Upjohn Company
Kalamazoo, Michigan

*TRADEMARK, REG. U. S. PAT. OFF.



Photos used with permission of patient.

Mrs. C.R. is Normotensive with Singoserp/Esidrix...

Relieved of hypertensive headache, patient can now carry out heavy responsibilities

Severe headache—a symptom of her hypertension—has troubled Mrs. C. R. for about 4 years. Her job and home life have imposed additional stress. Employed by a chocolate manufacturer—on the “swing shift”—she works in a cold room, wearing a coat and wool socks as protection. After work she waits a half hour for a bus that gets her home at 1:30 a.m.

Mornings at home offer no respite. Since her husband, a cardiac cripple, cannot help with

household chores, she does the cleaning and shopping, also works on the lawn and garden. Mrs. R. and her husband built their own house from the foundation up some years ago. After his incapacitating heart attack in 1957 she poured the concrete walks and patio herself.

Initially, Mrs. R.'s physician prescribed meprobamate and chlorothiazide, with no effect. On January 29, 1959, she was switched to Esidrix 50 mg. in combination with Singoserp 0.5 mg. daily;

Before treatment: B. P. 190/110 mm. Hg



her blood pressure was then 190/110 mm. Hg.

By March 9, Singoserp/Esidrix combination therapy had lowered Mrs. R.'s pressure to 150/100 mm. Hg. On June 1, the reading was 140/80 mm. Hg. As of August 24, the patient's blood pressure had stabilized at that normotensive level.

Mrs. R. is delighted with the results of Singoserp/Esidrix treatment. Her headaches are gone. She once again has the energy to handle

After treatment: B. P. 140/80 mm. Hg



her heavy responsibilities at work and at home.

With Singoserp-Esidrix you give your hypertensive patients the benefits of potentiated therapy. Often more effective than a single drug, Singoserp-Esidrix usually relieves hypertension without side effects. Indicated in mild to moderate hypertension.

SUPPLIED: Singoserp-Esidrix Tablets #2 (white), each containing 1 mg. Singoserp and 25 mg. Esidrix. Tablets #1 (white), each containing 0.5 mg. Singoserp and 25 mg. Esidrix.

Singoserp®-Esidrix®

(syrosingopine and hydrochlorothiazide CIBA)

Combination Tablets

POTENTIATED ANTIHYPERTENSIVE



CIBA
SUMMIT-NEW JERSEY



brightens life for the aged

NIAMID gives the depressed elderly person a new sense of well-being. The family will notice a sunnier outlook, an alert interest in group activities, a renewed awareness of personal appearance, and a return of appetite. Your patient will be more cooperative and less demanding.

You can expect to see the same excellent response to NIAMID in a wide variety of depressive syndromes—acute or chronic, mild or severe, whether associated with long-standing or incurable illness, or masquerading as organic disease.

NIAMID side effects are infrequent and mild, and often lessened or eliminated by a reduction in dosage. NIAMID has not been reported to cause jaundice, and significant hypotensive effects have rarely been noted.

DOSAGE: Start with 75 mg. daily in single or divided doses, and adjust according to patient response. NIAMID acts slowly, without rapid jarring of physical or mental processes. Some patients respond to NIAMID within a few days, but for full therapeutic benefit, most require at least two weeks. NIAMID is available as 25 mg. (pink) and 100 mg. (orange) scored tablets.

Already clinically proved in several thousand patients—

Complete references and a Professional Information Booklet giving detailed information on NIAMID are available on request from the Medical Department, Pfizer Laboratories, Division, Chas. Pfizer & Co., Inc., Brooklyn 6, N. Y.

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in geriatrics*

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 Science for the world's well-being™



women of
childbearing
age...
and growing
children...
may be



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Women of menstrual age and growing children have higher iron requirements than other individuals. Hence iron-deficiency anemias occur most often in these groups. Many clinicians recognize that most women need a hematinic for six weeks each year during reproductive years.

Livitamin, with peptonized iron and B complex, offers an excellent formula to restore depleted iron reserves in both adults and children. Peptonized iron is well absorbed and stored, and better tolerated than ferrous sulfate. B complex and other factors provide nutritional support.

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with Peptonized Iron

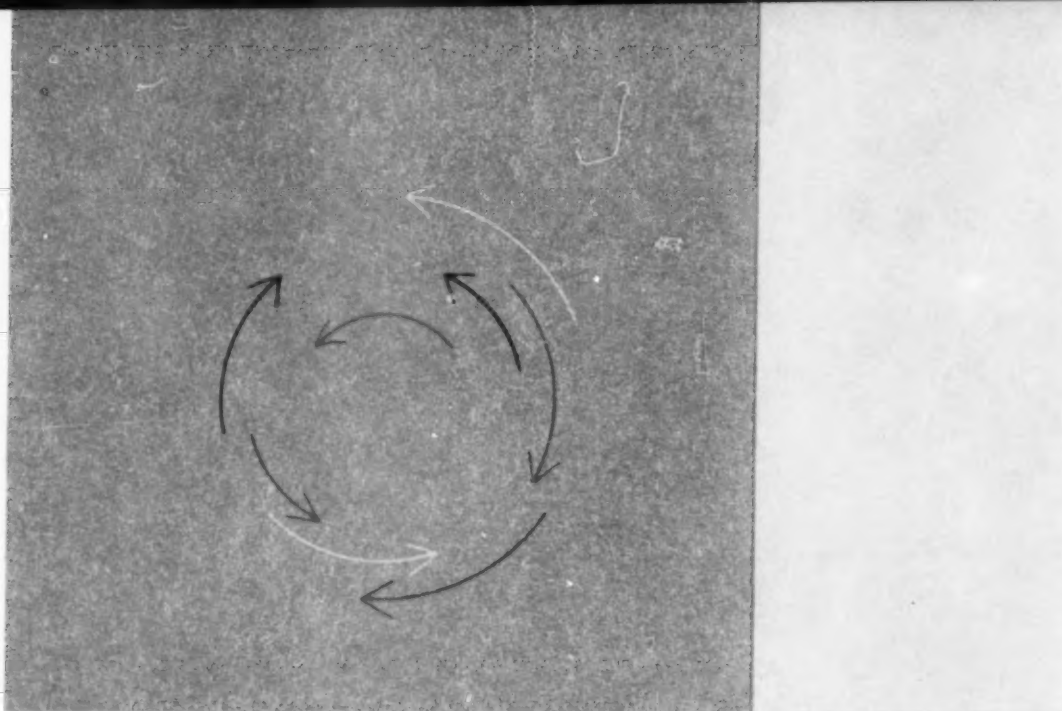
FORMULA: Each fluidounce contains:

| | |
|--------------------------------------|---------|
| Iron peptonized | 420 mg. |
| (Equiv. in elemental iron to 71 mg.) | |
| Manganese citrate, soluble | 158 mg. |
| Thiamine hydrochloride | 10 mg. |
| Riboflavin | 10 mg. |
| Vitamin B ₁₂ Activity | 20 mcg. |
| (Derived from Cobalamin conc.) | |
| Nicotinamide | 50 mg. |
| Pyridoxine hydrochloride | 1 mg. |
| Pantothenic acid | 5 mg. |
| Liver fraction 1 | 2 Gm. |
| Rice bran extract | 1 Gm. |
| Inositol | 30 mg. |
| Choline | 60 mg. |

SUPPLIED IN LIQUID OR CAPSULE.

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Livitamin assures patient acceptance because it is highly palatable. Peptonized iron provides a virtually predigested form of iron. Recent studies* show peptonized iron has these advantages:

- Rapid response in iron-deficiency anemias
- Non-astringent
- Absorbed as well as ferrous sulfate
- Better gastric toleration than ferrous sulfate
- Less constipating than ferrous sulfate



...the preferred hematinic

*Keith, J.H.: Utilization and Toxicity of Peptonized Iron and Ferrous Sulfate, Am. J. Clin. Nutrition 1:35 (Jan.-Feb., 1957).

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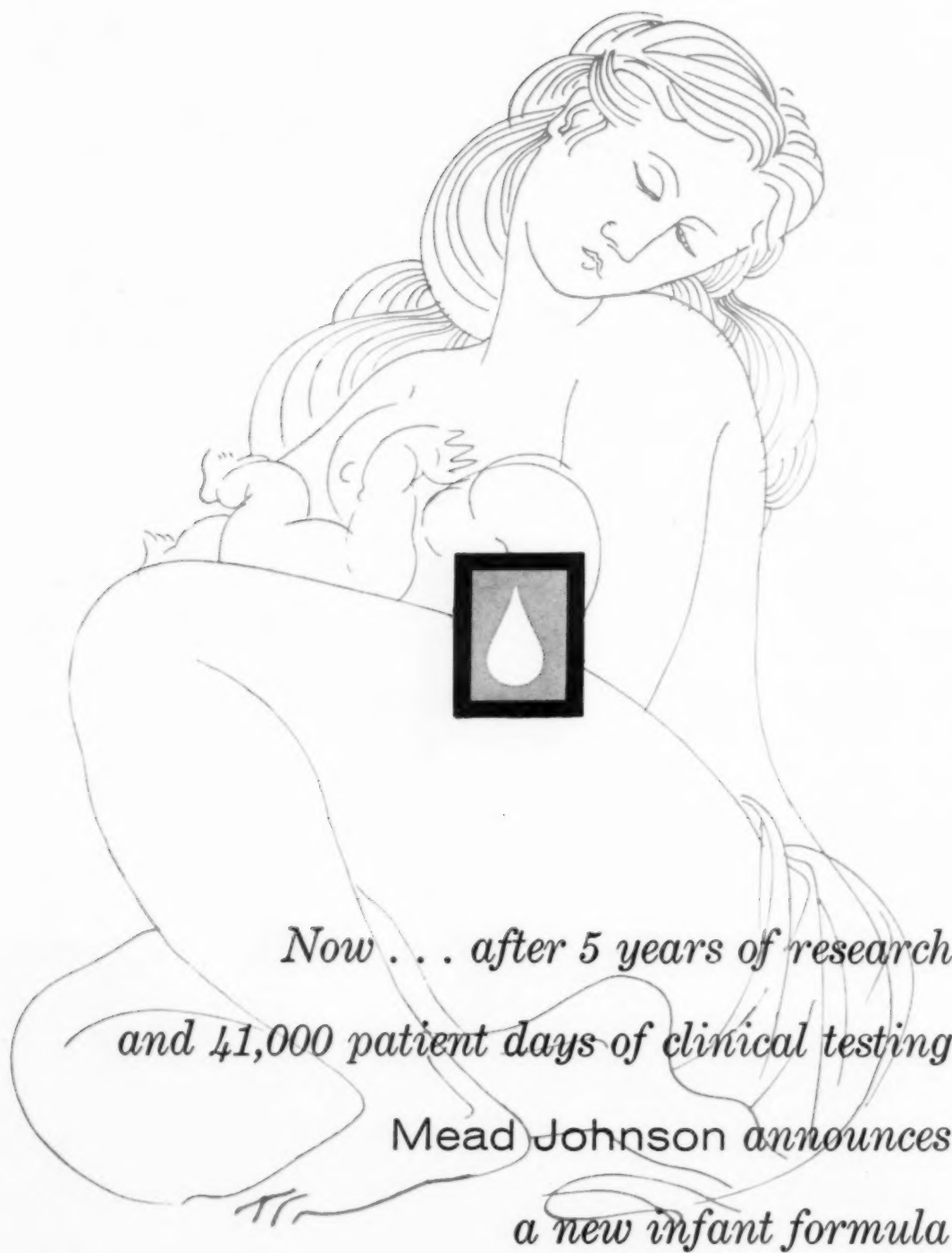
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in nutritional breadth and balance*

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in nutritional breadth and balance

In a well controlled institutional study², using the Latin Square technic[†] for the first time in infant nutritional research, Enfamil was compared with three widely used infant formula products.

This formula produced:

*weight gains greater than average
stool firmness between firm and soft . . . and
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NEAREST . . . to mother's milk in its pattern of protein, fat and carbohydrate by caloric distribution

NEAREST . . . to mother's milk in its pattern of vitamins and minerals (except for more vitamin D in accordance with NRC recommendations)

NEAREST . . . to mother's milk in its fat composition (no butterfat; no sour regurgitation)

NEAREST . . . to mother's milk in its ratio of saturated to unsaturated fatty acids

NEAREST . . . to mother's milk in its low renal solute load

ENFAMIL LIQUID—cans of 13 fluid ounces.

1 part Enfamil Liquid to 1 part water for 20 cal. per fl. oz.

ENFAMIL POWDER—cans of 1 lb., with measure.

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1. Macy, I. G.; Kelly, H. J., and Sloan, R. E., with the Consultation of the Committee on Maternal and Child Feeding of the Food and Nutrition Board, National Research Council: *The Composition of Milks*. National Academy of Sciences, National Research Council, Publication 254, Revised 1953. 2. Research Laboratories, Mead Johnson & Company.

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Lown, B., and Levine, S. A.: Current Concepts in Digitalis Therapy, Boston, Little, Brown & Company, 1954, p. 23, par. 2.

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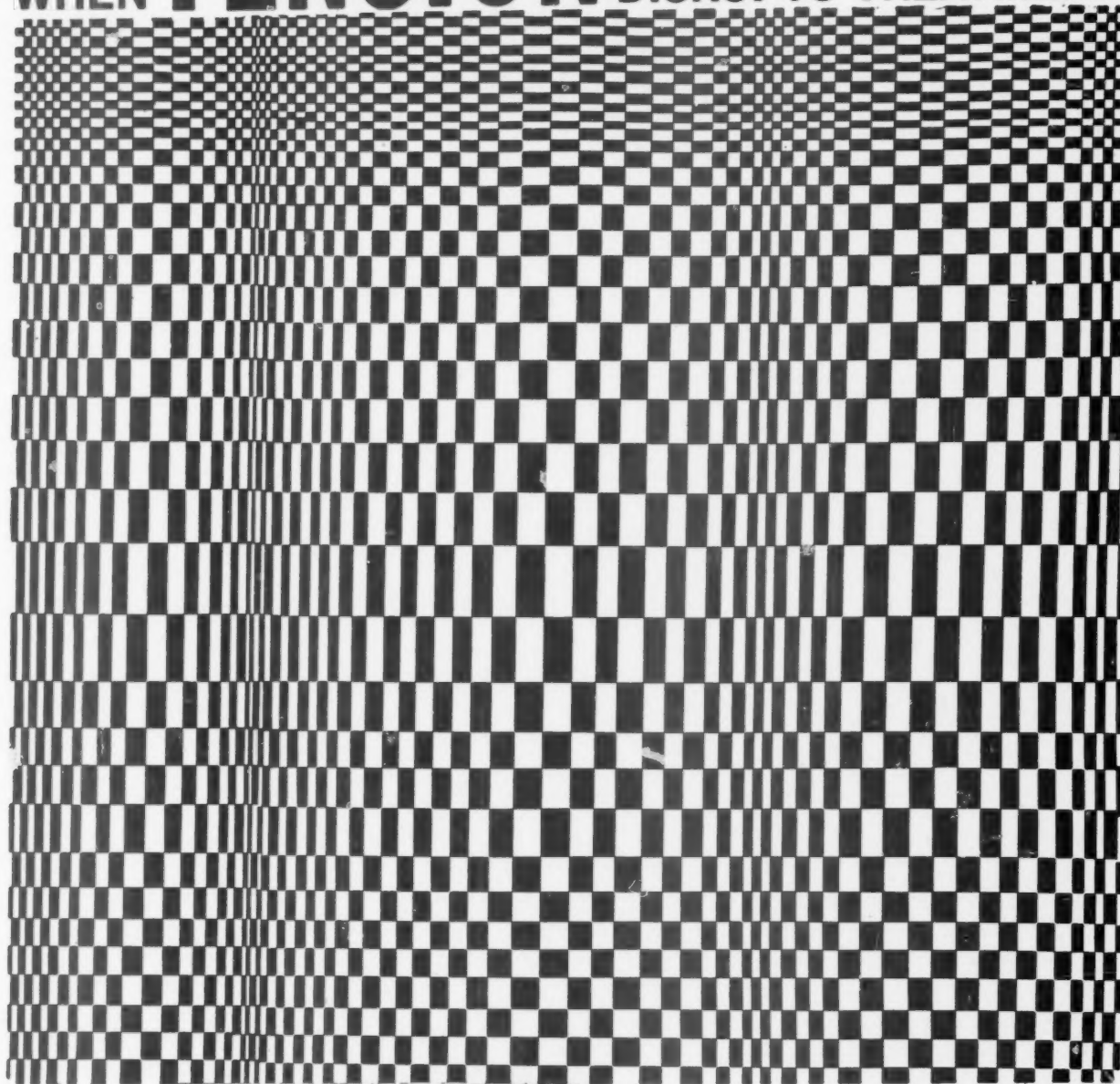
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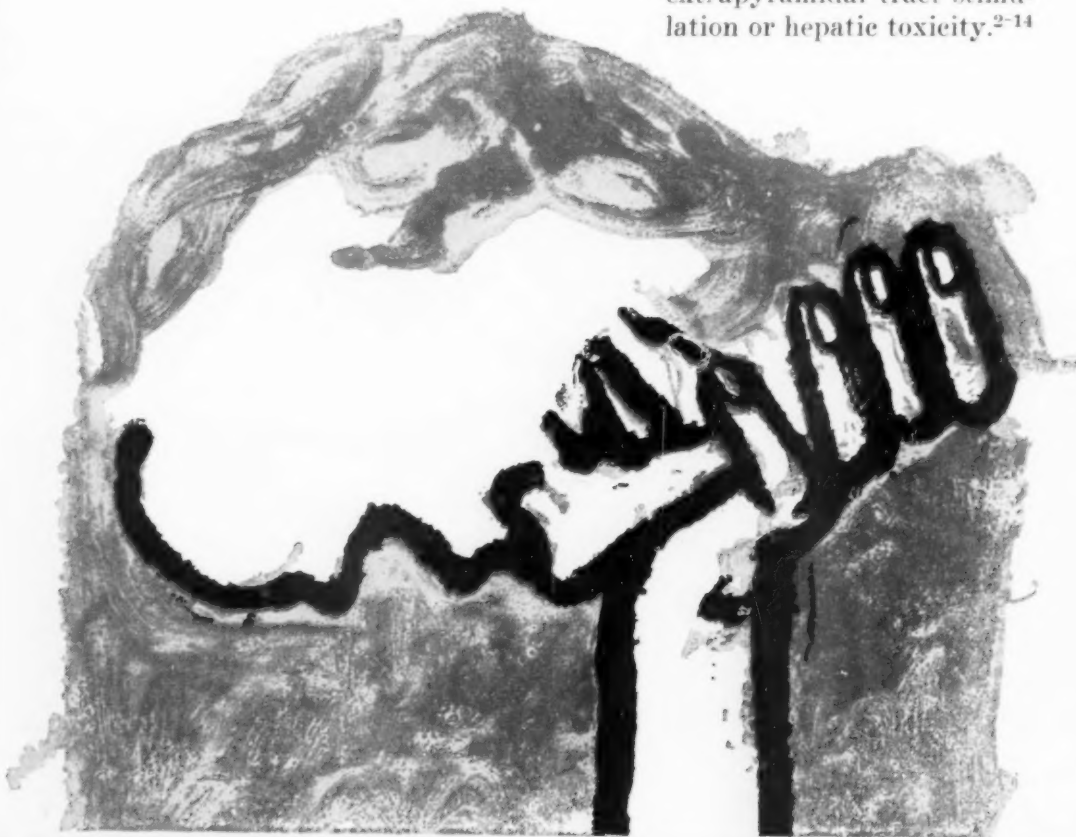
stop as well as prevent
nausea and vomiting

Tigan

now in oral,
parenteral, and
suppository forms

*effective but not
"side effective"*

Tigan blocks emetic impulses at the chemoreceptor trigger zone (CTZ),¹ a medullary structure activating the vomiting center. While Tigan shares with the phenothiazines the mode of antiemetic action, this is their only similarity.¹ In extensive clinical studies²⁻¹⁴ Tigan, unsurpassed in specificity, has exhibited a virtually complete absence of side effects. Tigan has demonstrated no sedative or tranquilizing properties, no hypotensive or supramedullary effects, no extrapyramidal tract stimulation or hepatic toxicity.²⁻¹⁴



no special precautions— no known contraindications

*in nausea/vomiting
of gastrointestinal
disorders*

Complete or moderate relief in 78 per cent of acute or chronic gastroenteritis patients;¹³ "We did not find a single toxic reaction . . . no side effects, such as sedation, skin rash . . . no changes in pulse, respiration, or . . . blood pressure."¹³

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of pregnancy*

No evidence of sedation or other side effects¹² observed in a series of patients of whom 94 per cent became asymptomatic on Tigan. On other antiemetic medication, several had failed to respond or had complained of drowsiness.¹²

*in nausea/vomiting
of radiation sickness*

Protected with Tigan ". . . not one patient had to discontinue [deep radiation] treatments. . . ."⁵

*in nausea/vomiting
of drug
administration*

" . . . large intermittent dose[s] of [nitrogen mustard and other drug] therapy could be given without the associated nausea and vomiting that we had seen before."⁷

Tigan

*specific
antiemetic
antinauseant*

*no sedative properties
no tranquilizer side effects*

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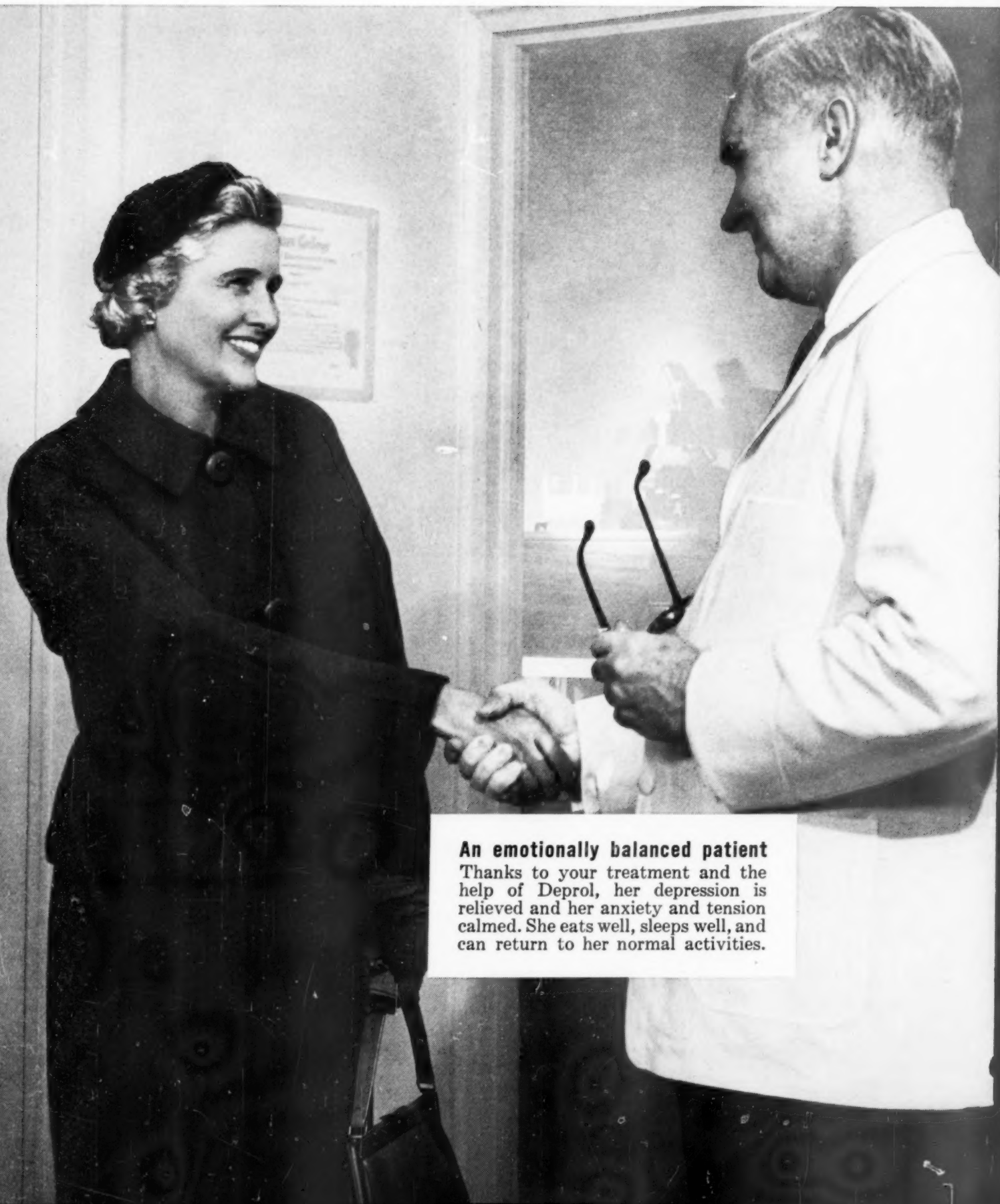
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References: 1. W. Schallek, G. A. Heise, E. F. Keith and R. E. Bagdon, *J. Pharmacol. & Exper. Therap.*, 126:270, 1959. 2. W. B. Abrams, I. Roseff, J. Kaufman, L. Goldman and A. Bernstein, to be published. 3. I. Roseff, W. B. Abrams, J. Kaufman, L. Goldman and A. Bernstein, *J. Newark Beth Israel Hosp.*, 9:189, 1958. 4. O. C. Brandman, paper read at Colloquium on the Pharmacological and Clinical Aspects of Tigan, New York City, May 15, 1959. 5. J. A. Lucinian, *ibid.* 6. D. W. Molander, *ibid.* 7. B. I. Snider, *ibid.* 8. W. S. Derrick, *ibid.* 9. B. Wolfson and F. F. Foldes, *ibid.* 10. L. McLaughlin, *ibid.* 11. Reports on file, Roche Laboratories. 12. Personal communications. 13. W. K. Gauthier, Discussant at Colloquium on the Pharmacological and Clinical Aspects of Tigan, New York City, May 15, 1959. 14. H. E. Davis, *ibid.*

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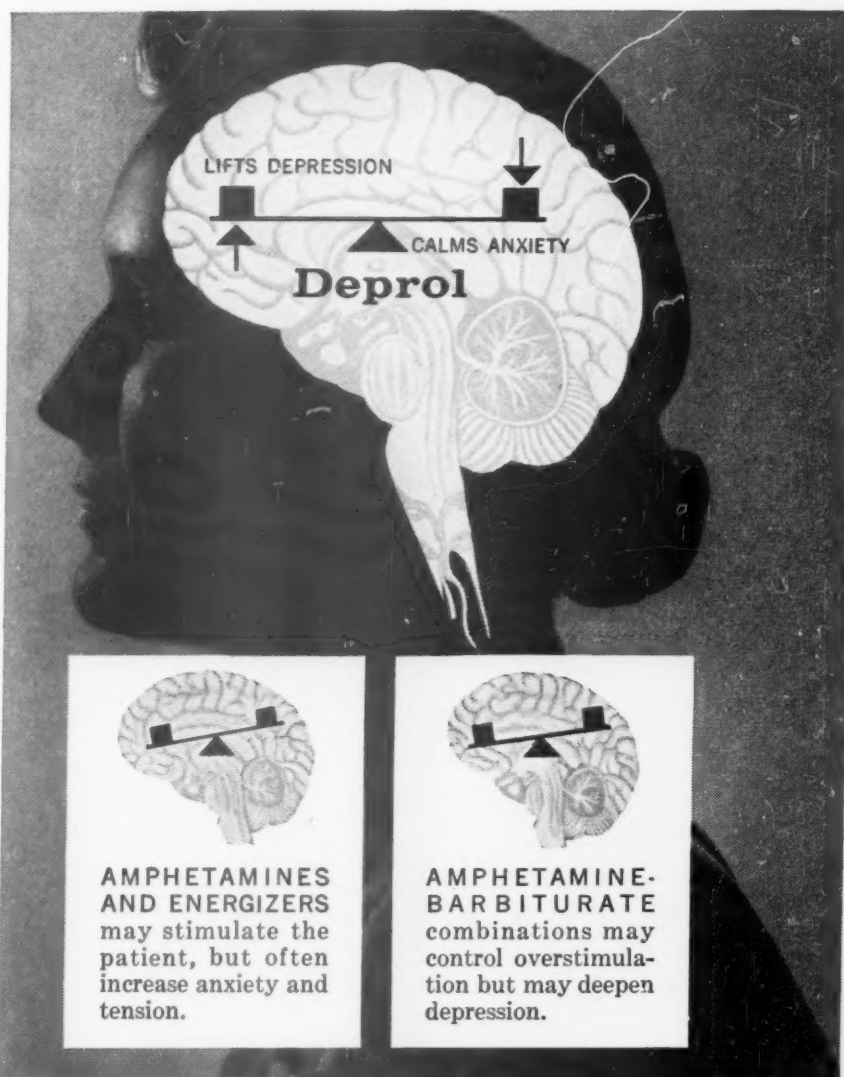
BIBLIOGRAPHY: 1. Alexander, L.: Chemotherapy of depression—Use of meprobamate combined with benactyzine (2-diethylaminoethyl benzilate) hydrochloride. *J. A.M.A.* 166:1019, March 1, 1958. 2. Bateman, J. C. and Carlton, H. N.: Deprol as adjunctive therapy for patients with advanced cancer. *Antibiotic Med. & Clin. Therapy*. In press, 1959. 3. Bell, J. L., Tauber, H., Santy, A. and Pulito, F.: Treatment of depressive states in office practice. *Dis. Nerv. System* 20:263, June 1959. 4. McClure, C. W., Papas, P. N., Speare, G. S., Palmer, E., Slattery, J. J., Konofal, S. H., Henken, B. S., Wood, C. A. and Ceresa, G. B.: Treatment of depression—New techniques and therapy. *Am. Pract. & Digest Treat.* In press, 1959. 5. Pennington, V. M.: Meprobamate-benactyzine (Deprol) in the treatment of chronic brain syndrome, schizophrenia and senility. *J. Am. Geriatrics Soc.* 7:656, Aug. 1959. 6. Rickels, K. and Ewing, J. H.: Deprol in depressive conditions. *Dis. Nerv. System* 20:364, (Section One), Aug. 1959. 7. Ruchwarger, A.: Use of Deprol (meprobamate combined with benactyzine hydrochloride) in the office treatment of depression. *M. Ann. District of Columbia* 28:438, Aug. 1959. 8. Settel, E.: Treatment of depression in the elderly with a meprobamate-benactyzine hydrochloride combination. *Antibiotic Med. & Clin. Therapy*. In press, 1959.

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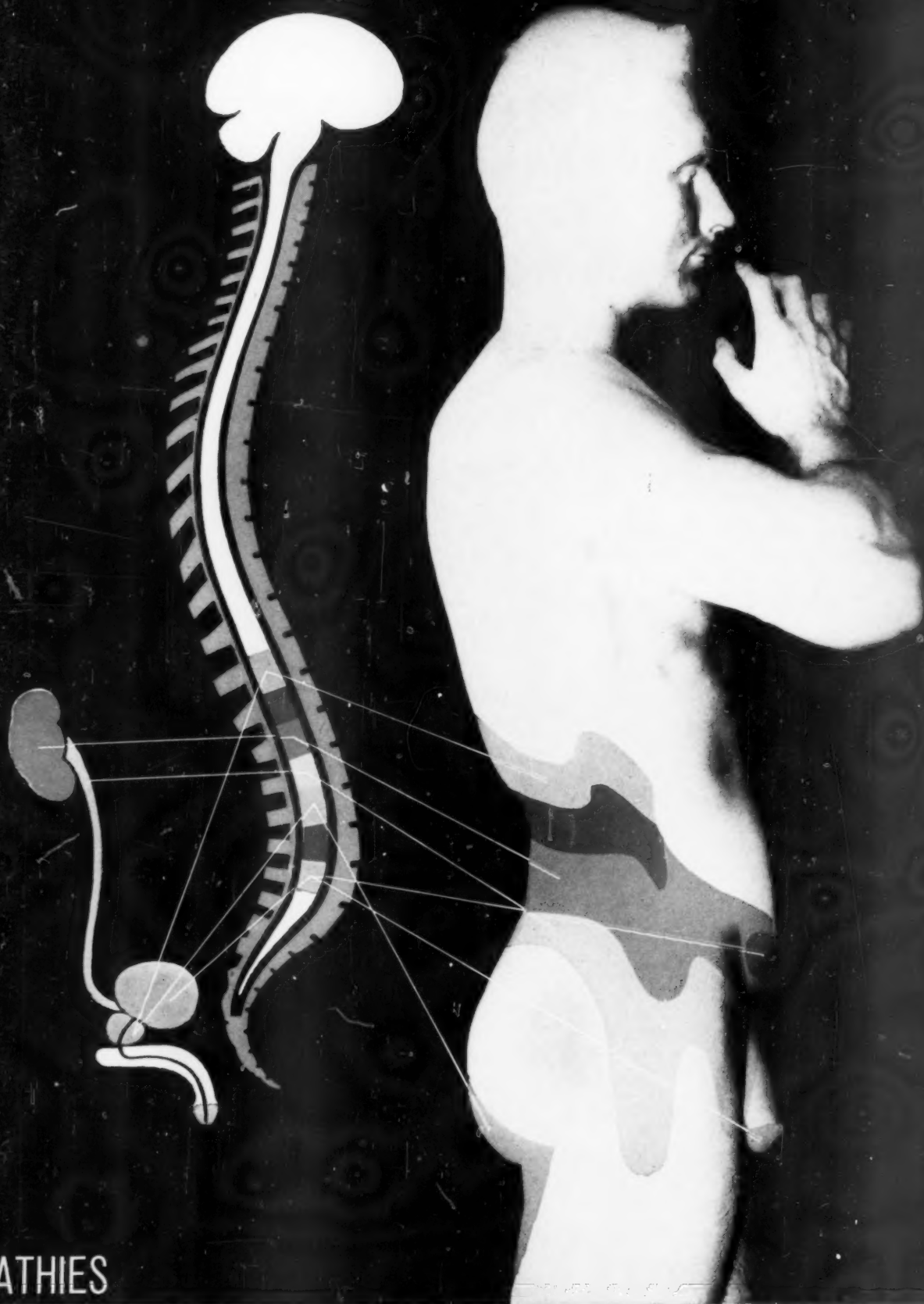


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AMPHETAMINE-BARBITURATE combinations may control overstimulation but may deepen depression.

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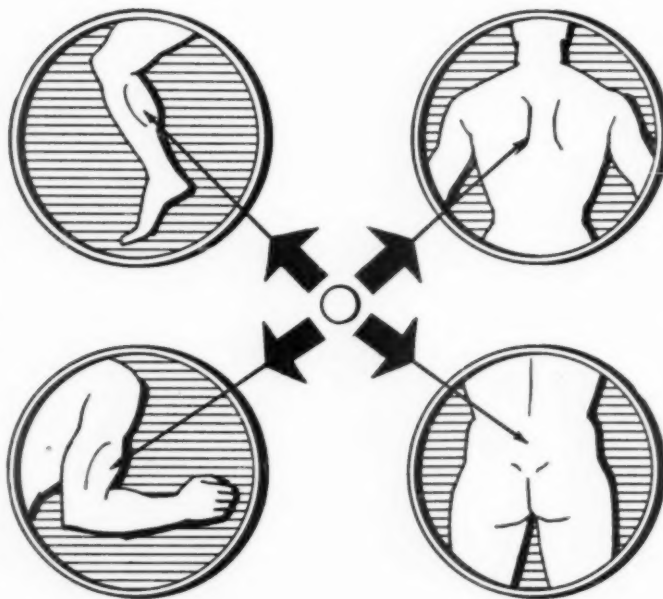
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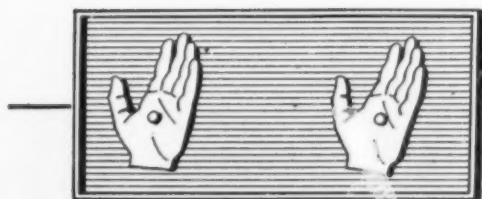
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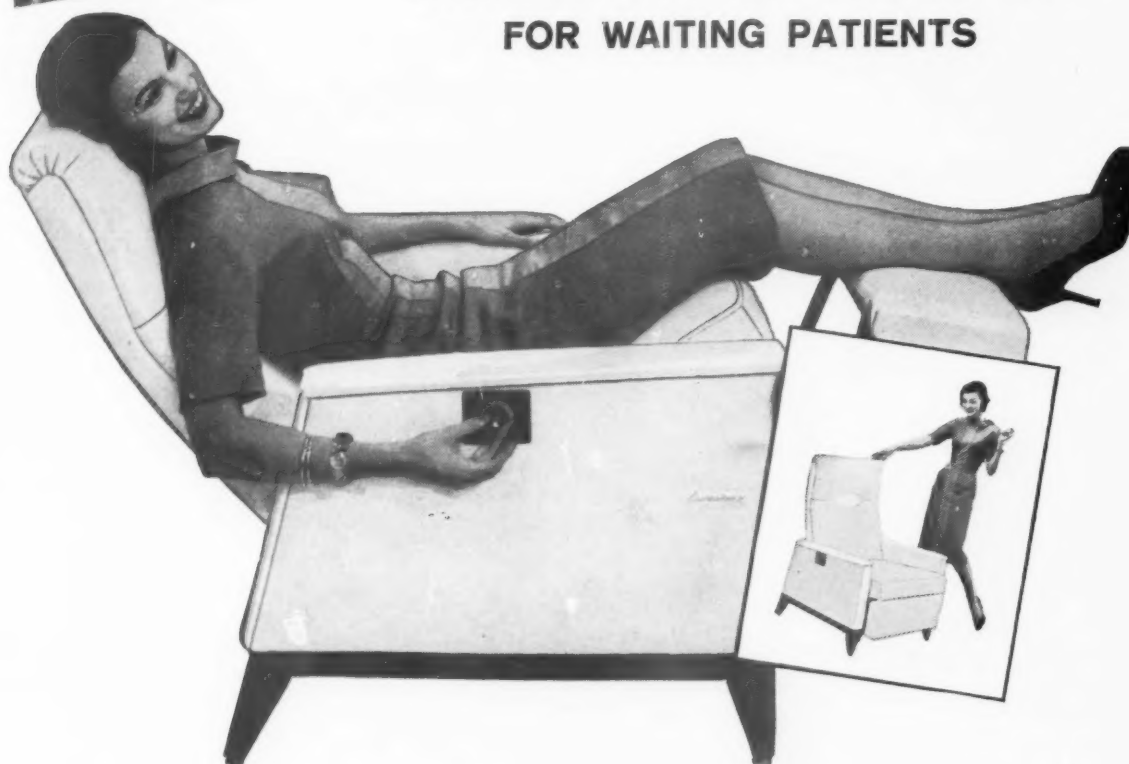
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References: 1. Cronk, G.A.; Naumann, D.E., and Casson, K.: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia Inc., 1958, p. 397. 2. Childs, A.J.: *Brit. M.J.* 1:660 (Mar.) 1956. 3. Newcomer, V.D.; Wright, E.T., and Sternberg, T.H.: *Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia Inc., 1955, p. 686. 4. Gimble, A.I.; Shea, J.G., and Katz, S.: *Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia Inc., 1956, p. 676. 5. Stone, M.L., and Mersheimer, W.L.: *Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia Inc., 1956, p. 862. 6. Campbell, E.A.; Prigot, A., and Dorsey, G.M.: *Antibiotic Med. & Clin. Ther.* 4:817 (Dec.) 1957.

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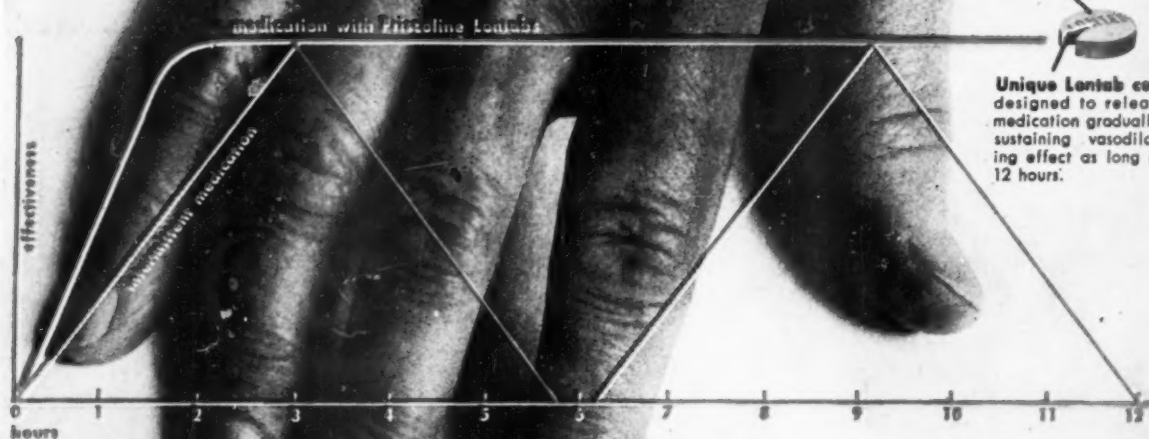
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